Food Research

An Official Publication of

The Institute of Food Technologists

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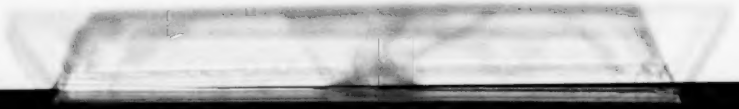

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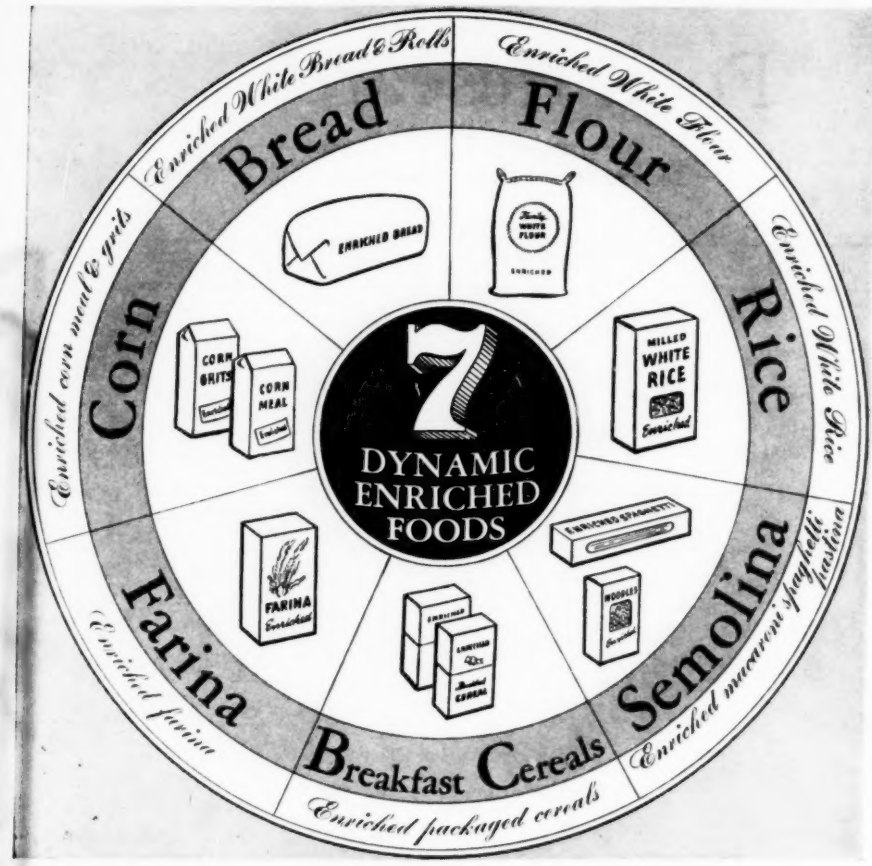

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A new and expanded edition of the pamphlet "Information and suggestions for authors" has been published for those writing technical contributions for *Food Technology* and *Food Research*.

The pamphlet discusses the respective scopes of the two journals and the type of articles suitable for them. A detailed outline is presented of the customary organization of technical papers followed by statements concerning the preferred styles and methods of preparing manuscripts. A discussion of various details like units of measurements and their abbreviations, use of footnotes and literature citations, preparation of tables and figures, etc. follows.

In the following chapter the customary methods of correcting printer's proofs are described. Here a list is given indicating the more common corrections. Instructions for the ordering of reprints follow.

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CHEMICAL CONSTITUENTS, PHYSICAL PROPERTIES, AND PALATABILITY OF FROZEN PORK*

ANDREA OVERMAN MACKEY, A. W. OLIVER, AND S. C. FANG
Departments of Home Economics, Animal Husbandry, and Agricultural Chemistry of the Oregon Agricultural Experiment Station, Oregon State College, Corvallis, Oregon.

(Manuscript received November 7, 1951)

This study was undertaken to determine the changes which occur during frozen storage of pork, particularly with reference to the chemical composition, physical properties, and palatability of pork stored at 0°F. (−18°C.) for approximately one year.

Changes that occur in lean meat during storage are chiefly enzymic while those that take place in the fat portion of the meat may be enzymic or autoxidative. If enzyme action proceeds at an appreciable rate, the following changes may be expected: free fatty acids as well as oxidation products of fat will increase; pH will decrease; breakdown products of proteins, such as amino acids and nitrogenous extractives, will increase; tenderness may increase and cooking time and cooking losses may decrease; and flavor changes will take place.

Enzyme action is retarded at freezing temperatures. Ball and Lineweaver *(4)* observed that at freezing temperatures proteolysis in three different protein-enzyme systems could not be detected. However, when the frozen protein materials plus enzymes were subsequently held at higher temperatures it became evident that changes had taken place during frozen storage. In contrast to the non-detectable changes in protein-enzyme systems when frozen, fat-enzyme systems showed that lipase action in the presence of unsaturated fats or the lower saturated fats proceeded at a measurable rate.

Hall and others *(11)*, in a study of chemical and palatability changes in frozen pork, found that peroxide numbers and free fatty acids were lower in pork stored for 6 months when held at −10°F.(−23.4°C.), and −20°F.(−28.9°C.) than in pork stored at 10°F.(−12.3°C.) or 0°F. (−18°C.). Little or no effect due to storage temperature was noted on shrinkage in storage or during cooking, on pH, on total or non-protein nitrogen in tissues, or in the percent press fluid.

At 0°F.(−18°C.), or lower, pork has been found to last 12 months without becoming rancid, whereas at temperatures of 10°-20°F.(−12.3°-−6.7°C.), rancidity has been noted after storage of 4 months *(8, 16, 5, 7,* and *18)*.

Though enzyme action would be slow at 0°F.(−18°C.), changes in frozen storage over a long period of time may be comparable to ripening changes at higher temperatures. If of sufficient magnitude, nutritive value and palatability may be affected.

*Published as Technical Paper No. 737 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution of the Department of Home Economics.

PROCEDURE

The hogs used for this study were Duroes and Berkshires. They had been fed a standard ration of ground wheat, meat meal, and Ladino pasture or alfalfa meal. They weighed an average of 210 pounds when slaughtered. After the carcasses were cooled for approximately 24 hours, the loins were removed and cut into 1-inch thick chops.

Chops from two groups of hogs were studied. Group one was composed of chops from Hogs A, B, and C. In this group all of the chops were wrapped in waxed locker paper. Group two was composed of chops from Hogs D and E. In this group, chops were wrapped in 3 different types of paper: cellophane-lined, waxed locker paper, and unwaxed paper.

Each chop was weighed and wrapped individually, then frozen. When types of wrapping paper were to be compared, neighboring chops were wrapped in the different papers. The right and left loins from each hog were handled in the same way; the left loin to be used for cooking tests, and the right loin to be used for chemical tests.

Tests were performed on fresh chops and on chops from each hog after monthly or bimonthly intervals of frozen storage up to 14 months. For chemical analysis, the lean and fat portions of the chops were separated. The lean was ground and thoroughly mixed. The fat was sliced, dried, and extracted. The extracted fat and the ground lean were stored at freezing temperatures until all analyses were complete. The following analyses were made on the lean of chops A, B, and C: pH, ash, fat, moisture, water-soluble and water-insoluble nitrogen, and total nitrogen *(3)*. Proteose nitrogen, coagulable nitrogen, and meat base nitrogen were determined on the cold water extract of the lean meat *(2)*. The following analyses were made on the fat of chops A, B, and C: saponification number, refractive index, iodine number, free fatty acids, soluble volatile acids, insoluble volatile acids *(3)*, peroxide oxygen *(15)*, and Kreis value *(19)*. The pH, ash, fat, moisture, and proteose nitrogen were determined on the lean, and peroxide number and Kreis value on the fat of chops D and E.

Chops to be cooked were thawed at 37.4°-41°F.(3°-5°C.) for 22 hours in individual tightly-covered cans. During thawing, each chop was suspended from a hook in the lid of the can so that the drip, if any, could be determined.

The chops were roasted in individual covered pans, equipped with racks, at a temperature of 253.4°-255.2°F.(123°-124°C.) until the interior temperature of the chops reached 185°F.(85°C.).

Objective measurements of tenderness were made by means of a shearing apparatus similar to the Warner-Bratzler machine. The percent of press fluid was calculated from the loss in weight of a 2.00-g. sample of lean meat, pressed at 8,000 p.s.i.g. for 5 minutes at a temperature of 104°F.(40°C.).

A panel of 5 judges scored the pork chops for the following factors: intensity and desirability of aroma, flavor of fat and lean, texture, and tenderness.

Analyses of variance were computed to determine the significance of differences for various factors due to storage time, hog, or wrapping paper. Correlation coefficients were calculated for shrinkage during storage, press fluid, and cooking losses.

RESULTS

Physical qualities. Moisture was lost from the pork chops during frozen storage, Table 1. Chops from hogs D and E yielded the following information: those wrapped in unwaxed paper lost most moisture, those wrapped in cellophane-lined paper least, and those wrapped in waxed locker paper were intermediate in weight loss. These differences were highly significant, as shown by the analysis of variance, Table 2. When the wrapper allowed an appreciable evaporation of moisture, weight loss increased as storage time progressed. When wrapping papers were effective in preventing moisture loss, the length of time in frozen storage had little influence on the percent of weight lost. The pork chops lost no moisture or juice during thawing. Sair and Cook *(17)* reported little drip loss in pork and noted that pork and mutton usually have a more alkaline pH than other meats.

Their findings showed maximum drip at pH 5.2 with a decrease to no drip at pH 6.4. The pork chops herewith reported had pH values ranging from 5.46 to 7.50 with an average pH value of 6.00.

TABLE 1

Average shrinkage during storage, cooking losses, press fluid, and shearing strength of pork chops stored up to 14 months at 0°F.(—18°C.)

Type of wrapping paper	Pork chop groups	Storage losses	Cooking losses	Press fluid	Shearing strength
		%	%	%	*pounds*
Waxed	A	1.5	17.2	40.4	17.0
	B	1.8	16.4	42.2	15.0
	C	1.2	15.9	43.0	16.3
Waxed	D	1.3	14.4	50.7	27.8
	E	1.3	15.4	47.6	28.4
Unwaxed	D	7.9	10.2	51.9	27.9
	E	6.8	12.3	46.8	28.1
Cellophane-lined	D	0.7	16.6	48.8	33.1
	E	0.7	17.4	46.7	27.1

TABLE 2

Analyses of variance of shrinkage during storage, cooking losses, and press fluid of pork chops stored at 0°F.(—18°C.) up to 14 months mean squares and significance of differences [b]

Pork chop groups	Variation	D.F.	Shrinkage	Cooking loss	Press fluid
A, B, C	Time of storage	9	1.0437*	13.4302—	38.1876—
	Hogs	2	0.7410*	4.7305—	19.4413—
D, E	Time of storage	13	1.59—	22.72*	63.212*
	Types of wrap	4	76.535*	65.088*	10.25—

[b] * inidcates a significant difference; — indicates no significant difference.

Moisture was lost from the pork chops both during frozen storage and during cooking. These losses tended to balance each other. Though moisture was conserved by good wrapping paper it tended to be lost in cooking. Cooking losses averaged 17.0% for chops which had been wrapped in cellophane-lined paper, 14.9% for those wrapped in waxed locker paper, and 11.3% for those wrapped in unwaxed paper, Table 1. The outside of chops wrapped in unwaxed paper became so desiccated that after several months of storage only a drop or two of juice was obtained during cooking. If the dried layer were trimmed off, the inside meat was found to compare favorably in flavor and juiciness with the chops wrapped in other papers which delayed or prevented surface drying of the meat.

The variations in cooking losses due to hog were not great, though the differences between hogs in cooking losses were greater than the differences in storage losses. Different lots of chops wrapped in waxed locker paper averaged 14.4 to 17.2% cooking loss, Table 1.

The analysis of variance revealed no difference due to type of wrapping paper in the percent of press fluid from frozen chops, Table 2. There was a slight tendency toward increased press fluid with longer storage, the correlation coefficient between storage time and percent press fluid being +0.6903. The reason for this is not apparent. A decrease in pH values may partly account for an increase in press fluid in frozen, stored meat. On the other hand, chops from different hogs yielded different amounts of press fluid. These variations appeared to be unrelated to the pH of the meat.

As has been mentioned, cooking losses varied inversely with evaporation loss during storage. This was particularly evident for chops that had been wrapped in different types of paper. Yet press fluid was not significantly different between these groups of chops. Hence it appears that there is a maximum amount of moisture which can readily be lost from lean pork. The moisture lost during storage and also during cooking is loosely held, whereas moisture remaining in the meat after it has been thoroughly cooked is more tightly held. Empey *(10)* has suggested that a certain fraction of the muscle fluid, which he found could be easily expressed, was probably held by capillary attraction in the muscle fiber.

A shorter cooking time or more rapid heat penetration may accompany enzyme action or ripening of meat. Hanson, Stewart, and Lowe *(12)* found that cooking losses and cooking time for poultry decreased as the time between killing and cooking increased. Alexander and Clark *(1)* reported that as lamb was ripened longer it shrank relatively less during roasting and cooked faster in proportion to its weight.

When cooked under uniform conditions at a low oven temperature the time required for 1-inch thick pork chops to reach an interior temperature of 185°F.(85°C.) was approximately 1 hour and 50 minutes regardless of the length of time the chops had been held in frozen storage. In preliminary trials it was demonstrated that when chops were roasted for a 2-hour period, the interior temperature attained was not influenced by the length of time the chops had been frozen. Hence, the shortened cooking time or more rapid heat penetration which might be expected to accompany enzyme action was not observed in frozen pork.

Chops from different hogs varied in shearing strength. Neither the period of time in frozen storage nor the type of wrapping paper had a consistent effect.

A relationship may exist between shearing strength and fat content of the lean. Hogs D and E, which had a low percentage of fat in the lean, averaged highest in shearing strength. The pH values, which averaged from 5.57 to 6.39 had no apparent relationship to differences in shearing strength.

Palatability scores. The analyses of variance for palatability scores are shown in Table 3.

The mean scores for all palatability characteristics were significantly different due to time of storage. However, the plotted data revealed no trends except in the following cases, in which scores tended to decrease as the storage period increased: tenderness of chops from Hog D; desirable flavor of fat of all groups of chops; desirable flavor of lean of chops of groups D and E.

TABLE 3

Analyses of variance of palatability scores of pork chops stored at 0°F.(—18°C.) up to 14 months mean squares and significance of difference [c]

Pork chop groups	Variation	D.F.	Intensity					Desirability		
			Texture	Tender-ness	Flavor of fat	Flavor of lean	Aroma	Flavor of fat	Flavor of lean	Aroma
A, B, C	Time of storage	10	5.5576—	3.9515—	15.6546*	15.7333*	15.8727—	37.8727*	12.3394—	11.1333—
	Hogs	2	0.7576—	3.3035—	0.6364—	0.3637—	5.3031—	5.1212—	3.8485—	0.8485—
D	Time of storage	7	24.2798*	90.1905*	9.8036—	6.8036*	13.6905*	51.1429*	18.5179*	14.8512*
	Type of wrap	2	0.5000—	1.0417—	0.2917—	0.1667—	2.6667—	31.2917*	1.0417—	3.1250—
E	Time of storage	6	5.2556*	40.7667*	6.6334—	3.4667—	24.7556*	37.5556*	7.0222*	14.4000*
	Type of wrap	2	1.5555—	0.5005—	3.5000—	2.1667—	5.0556*	12.3889—	1.5556—	3.5000—

[c] See footnote to Table 2.

The type of wrap had some influence on aroma and on flavor of fat. Chops wrapped in unwaxed paper tended to have more intense aroma and the flavor of the fat was less desirable than that of chops wrapped in waxed locker paper or cellophane-lined paper.

The flavor of the fat deteriorated gradually as shown by lower scores with increased length of storage period. This loss of desirability was due to: a, absorption of odors from the locker, especially by chops wrapped in unwaxed paper; and b, enzyme action or absorption by the fat of oxygen. Off-flavors were noted by some judges in chops stored 2 months, though flavor was not generally considered very objectionable except in chops stored for 8 months or more. The flavor of the lean meat tended to lose desirability as frozen storage was prolonged more than 8 months. Emmett and Grindley *(9)* state that the nitrogenous organic extractives contribute in part to the flavor of cooked meats. Hiner, Gaddis, and Hankins *(11)* observed a progressive decrease in soluble protein nitrogen in frozen lean tissue and ascribed this decrease to denaturation. They state that, although protein denaturation may be related to loss in quality of the lean, it is an unimportant factor in comparison with influence of the fat. They concluded that oxidation of the fat was chiefly responsible for decline in quality of freezer-stored meats.

In the study herewith reported, neither the organic extractives nor other chemical constituents of the lean changed appreciably, hence the less desirable flavor noted was apparently not due to chemical changes during frozen storage of the lean tissue.

The proportion of fat embedded in the lean of pork is usually higher than that in other meat. This close association of fat and lean in pork could possibly account for the off-flavor observed in the lean. Though the fat content averaged from 3.0 to 14.7% in different groups of chops, the flavor change of the lean was no faster for chops having a high percent of fat in the lean than for those having a low percent.

Chemical composition. The chemical constituents of the lean showed little change in pork chops which were stored at 0°F.(−18°C.) up to 14 months, Table 4. A decrease in pH values was observed the first 4 months of frozen storage. No further decrease took place throughout succeeding months. The decrease may have been due to a slow accumulation of lactic and other acids since lowered pH values have been found to coincide with increases in lactic acid in beef, pork, and other meats *(6)*.

The pH varied more between hogs than within hogs. For example, chops from hog D had comparatively high pH values, averaging close to 7.0 when fresh and 6.1 after storage. Pork from all other hogs tended to have lower pH values. Hog E had a pH value close to 6.0 when fresh and 5.5 after frozen storage for 4 months. The pH values for hogs A, B, and C after storage at 0°F.(−18°C.) for 14 months were 5.75 to 5.94. These values are somewhat higher than those usually reported, the normal reserve of glycogen in a fully rested pig at death giving an ultimate pH of 5.4 to 5.6 according to Callow *(6)*.

The pH values of the chops undoubtedly influenced a number of the properties of the meat. For example, there was no drip from the chops

TABLE 4

Chemical and physical properties of the lean of pork chops fresh and after storage at 0°F.(—18°C.) for 14 months

Pork chop groups	Storage period	Type of wrapping paper	Lean, natural basis									
			pH	Ash	Fat	Moisture	Nitrogen					
							Cold-water extract			Lean		
							Protease	Coagulable	Meat base	Water soluble	Water insoluble	Total
	months			%	%	%	%	%	%	%	%	%
A	0	None	.....	1.06	11.69	67.35	0.02	0.29	0.31	0.62	2.51	3.13
	14	Waxed	5.72	1.07	12.26	65.00	0.03	0.37	0.31	0.71	2.63	3.34
B	0	None	.....	1.06	9.15	69.05	0.04	0.29	0.30	0.63	2.70	3.35
	14	Waxed	5.75	1.16	8.41	70.17	0.04	0.36	0.40	0.74	2.69	3.43
C	0	None	.....	0.95	14.71	64.71	0.01	0.33	0.32	0.66	2.46	3.12
	14	Waxed	5.94	1.06	10.41	66.44	0.03	0.36	0.37	0.76	2.53	3.29
D	0	None	6.81	1.24	4.58	73.37	0.40	.....	.....	.....	.....	.....
	14	Unwaxed	6.04	1.32	8.48	63.98	0.10	.....	.....	.....	.....	.....
	14	Waxed	6.22	1.13	7.29	69.78	0.11	.....	.....	.....	.....	.....
	14	Cellophane	6.17	1.23	9.38	67.32	0.12	.....	.....	.....	.....	.....
E	0	None	6.12	1.12	7.18	70.35	0.08	.....	.....	.....	.....	.....
	14	Unwaxed	5.59	1.20	8.58	65.39	0.11	.....	.....	.....	.....	.....
	14	Waxed	5.61	1.14	6.22	68.99	0.11	.....	.....	.....	.....	.....
	14	Cellophane	5.56	1.11	6.90	69.56	0.11	.....	.....	.....	.....	.....

TABLE 5

Chemical and physical properties of the fat of pork chops, fresh and after storage at 0°F.(—18°C.) for 14 months

Pork chop groups	Storage period	Type of wrapping paper	Oxidizability value	Saponification number	Refractive index	Free fatty acids	Soluble volatile acid	Insoluble volatile acid	Peroxide oxygen no.[a]	Kreis value[b]	Iodine no. Hanus
	months										
A	0	None	2.40	196.6	1.4588	0.52	0.57	0.44	0.28	10.3	57.8
	14	Waxed	.....	197.7	1.4588	0.53	0.29	0.13	2.25	46.8	59.3
B	0	None	2.84	196.0	1.4585	0.55	0.54	0.41	0.26	10.0	54.8
	14	Waxed	.....	197.8	1.4585	0.48	0.27	0.10	4.72	56.2	55.9
C	0	None	3.45	196.3	1.4586	0.46	0.59	0.16	0.29	8.7	57.2
	14	Waxed	.....	197.0	1.4587	0.42	0.28	0.09	3.22	49.7	58.0
D	0	None	.....	.....	.....	.....	.....	.....	0.14	2.6	.....
	14	Unwaxed	.....	.....	.....	.....	.....	.....	1.20	7.0	.....
	14	Waxed	.....	.....	.....	.....	.....	.....	1.21	9.0	.....
	14	Cellophane	.....	.....	.....	.....	.....	.....	1.08	9.0	.....
E	0	None	.....	.....	.....	.....	.....	.....	0.22	0.8	.....
	14	Unwaxed	.....	.....	.....	.....	.....	.....	0.64	9.4	.....
	14	Waxed	.....	.....	.....	.....	.....	.....	0.81	12.0	.....
	14	Cellophane	.....	.....	.....	.....	.....	.....	1.20	13.4	.....

[a] No. of cc. of 0.002 *N*. $Na_2S_2O_3$ per gram of fat.
[b] Klett units per gram of fat, using number 54 filter.

during thawing. Sair and Cook *(17)* have pointed out that as the pH increases the net drip decreases to zero at about 6.4. Meat is also probably at its least tender point when at pH values of 5.0 to 6.0 according to Winkler *(20)*.

The physical and chemical properties of the fat portion of frozen pork chops remained practically constant throughout storage, Table 5, except for peroxide number and Kreis number, which showed a gradual increase. This indicated that oxidation of the fat took place continuously throughout the storage period. The high percentage of unsaturated acids contained in pork fat *(13)* would account for this trend. Yet values for these tests were too low, even after 14 months of frozen storage, to indicate rancidity in fat. The flavor deterioration, as shown by judges' scores, was more marked than was the chemical change.

SUMMARY AND CONCLUSIONS

Few changes took place during frozen storage of pork. The one of most practical significance was the gradual loss in desirable flavor of fat. This was accompanied by an increase in peroxide number and Kreis number. Tenderness remained similar to that of fresh pork. The time required for cooking chops to a given internal temperature remained approximately the same throughout storage, and cooking losses were correlated with surface drying rather than with length of storage.

The chemical constituents of the lean remained practically constant throughout storage at 0°F.(−18°C.) no trends being noted toward an increase in products of protein hydrolysis or toward a decrease in soluble protein constituents due to denaturation.

Hence, it must be concluded that frozen pork when properly wrapped and stored at 0°F.(−18°C.) for approximately one year retained essentially all of its identity as fresh pork.

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NITROGEN COMPOUNDS OF COFFEE[a]

G. E. UNDERWOOD AND F. E. DEATHERAGE
Department of Agricultural Biochemistry, Institute of Nutrition and Food Technology, The Ohio State University, Columbus, Ohio

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Comparatively little fundamental chemical knowledge about coffee is available even though this food is widely used and is of great economic importance. Consequently, there is no general agreement regarding the changes that take place in the roasting process. The chemical compounds responsible for the desirable aroma and flavor of roasted coffee are not definitely established; neither is it understood why roasted coffee becomes stale. The recent introduction of soluble coffee extracts on the market has stimulated fundamental research on coffee with a view to solving these problems.

In any product where odor and flavor are as important as they are in coffee, it would be expected that nitrogen-containing compounds would play a major role. The three most important sources of nitrogen in the raw coffee seed or "bean" are caffeine, trigonelline, and protein. Caffeine has been thoroughly investigated and trigonelline has recently been the subject of some comprehensive work, especially by Slotta and Neisser *(4)* and by Hughes and Smith *(3)*. However, the protein fraction has apparently not been critically examined, even though it is often reported that green coffee contains around 10% protein. Therefore, it appears that a study of the nitrogen compounds of the coffee bean, with emphasis on the protein fraction, would contribute to a more complete understanding of the chemical composition of coffee.

Since no previous work on the protein fraction of coffee has been reported, it was necessary to carry out several general experiments to learn something of the nature of the proteins present in the green coffee bean. These preliminary experiments were conducted on two different coffees, Santos and Medellin Excelso (Colombian). Coffees are divided into two major groups, Brazils and Milds. Santos is considered the best of the Brazils, while the Colombian coffees are the most widely used of the Mild group. Mild coffees are generally considered to have more acidity, and a more desirable aroma and appearance.

EXPERIMENTAL

First, the proteins of the green coffee bean were classified with respect to the traditional solubility groups, as set forth by the Committee on Protein Nomenclature *(1)*. Samples of the green beans, ground in a hammer mill to approximately 40-mesh size, were repeatedly extracted with water by mixing thoroughly, centrifuging, and filtering the supernatant liquid on suction. The combined extracts were treated with an equal volume of a 5% aqueous solution of trichloracetic acid *(2)*; this reagent precipitates proteins, but not proteoses, peptones, etc. The precipitated protein was successively washed with alcohol and ether, dried 1 hour at 105°C., then cooled and weighed.

[a] This material is taken from a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Ohio State University.

Similar extractions were carried out using the following solvents rather than water: a, 80% ethanol; b, 10% sodium chloride; and c, 1% sodium hydroxide. To obtain approximate values for the amount of protein extracted by each solvent, it was assumed that the precipitate was all protein. The percentage of protein was calculated in terms of the dry green coffee (Table 1). The moisture content of both green and roasted coffee samples was determined by drying for 2 hours in a vacuum oven at a temperature of 100°C. and a pressure of 4 inches of mercury.

TABLE 1

Amount of soluble protein present in green coffee

Sample	Extracting liquid	Protein
		%
Colombian	80% ethanol	0
Santos	80% ethanol	0
Colombian	Water	2.9
Santos	Water	3.0
Colombian	10% NaCl	3.0
Santos	10% NaCl	3.1
Colombian	1% NaOH	4.0
Santos	1% NaOH	4.9

A sample of the water-soluble protein was isolated as follows: 50 g. of the ground green coffee was vigorously shaken with 400 ml. of water at room temperature. The mixture was centrifuged and filtered on suction. To 300 ml. of the filtrate was added, all at once, 6 ml. of concentrated hydrochloric acid. After stirring thoroughly, the mixture was allowed to stand for 5 minutes. The protein precipitated immediately and settled rapidly. The supernatant liquid was decanted, the residue centrifuged, and again the liquid was decanted. The residue was washed 5 times with 95% ethanol (total volume of 300 ml.), centrifuged after each washing, and the wash liquid discarded. The residue was then washed 3 times with ethyl ether, filtered on suction, and washed twice with ether while on the filter. After drying for 1 hour at 105°C., the protein was finely ground in a mortar, then continuously extracted for 15 hours with absolute ether. The material was then dried for 15 hours at 105°C. There was obtained 0.7-0.8 g. of solid; the Colombian was nearly white, the Santos slightly brown in color. The nitrogen content of the sample prepared from the Colombian beans was 15.42%, while that from the Santos was 15.38%.

To further characterize the water-soluble proteins, the isoelectric point of the proteins was determined. Samples of the green coffee were extracted with water as described above, but in this instance the protein was precipitated by cooling the extract to 0°C. and then pouring it into 5 volumes of 95% alcohol, also cooled to 0°C. After standing overnight in the refrigerator at 8°C., the solid which had separated was filtered off, washed successively with cold alcohol and cold ether, then continuously extracted with absolute ether. After drying in the vacuum oven, the brownish powder had a nitrogen content of 9.0%. Assuming that the nitrogen was present as protein, this meant that the sample was about 60% protein. The entire sample was readily soluble in water, indicating that the proteins had not been denatured in the isolation procedure. The solution gave a voluminous precipitate on addition of trichloracetic acid.

Approximately 1% aqueous solutions of this protein concentrate were prepared from both the Colombian and Santos coffees. Equal aliquots of each solution were pipetted into separate series of standard acetate buffers. By observing the pH of the buffers in those tubes in which the protein was precipitated most rapidly and most completely, the isoelectric point of the water-soluble proteins prepared from both coffees was found to be at pH 4.6-4.7.

Experiments were designed to measure the activity of any proteolytic enzymes present in the water extracts of the green coffee beans. A water extract was prepared by extracting 50 g. of green coffee with 500 ml. of distilled water. The extract was adjusted to pH 7 by addition of solid sodium carbonate. Separate portions of the extract were saturated with toluene and incubated at various temperatures. By adding toluene or sodium benzoate (to prevent bacterial action) to the green coffee extract, it was possible

to follow the enzymic decomposition of the protein by merely running an occasional amino nitrogen determination, according to the procedure suggested by Van Slyke *(5)*. An increase in amino nitrogen would indicate that proteolysis was occurring and that a protease was present. Results of these determinations are reported in Table 2.

TABLE 2

Measure of activity of proteolytic enzymes

Sample	Temperature	Preservative	Volume of N_2 per ml. after			
			0 hrs.	36 hrs.	111 hrs.	200 hrs.
	°C.					
Colombian	25	Toluene	0.20	0.21	.....	0.20
Colombian	37	Toluene	0.20	0.19	0.18	0.18
Santos	25	Toluene	0.21	0.21	.....	0.21
Santos	37	Toluene	0.21	0.20	.....	0.21
Santos	25	Sodium benzoate	0.21	0.21	0.19	0.21

To further check the stability of the soluble coffee proteins, the amount of protein precipitated from an extract by trichloracetic acid was measured. A portion of the same extract was stored under toluene and another portion of the extract placed in a stoppered flask without preservative. The amount of protein precipitated from these two samples after standing several hours was compared with the amount of protein originally precipitated from the extract. It was found that the amount of precipitate obtained from the extract containing toluene was essentially constant over a period of 10 days; however, in the toluene-free extract, all of the protein had disappeared in 2 days.

To establish a basis for further work, it was considered necessary to divide the nitrogen compounds of the coffee bean into smaller groups. Since it had already been demonstrated that an appreciable part of the protein fraction could be extracted from the green beans, the decision was made to use solubility as the basis for securing partial separation of the nitrogen compounds. This work was confined to the Santos coffee. This represents the class of greatest commercial importance and it was felt that more could be accomplished in these investigations if only a single type of coffee was studied. The following experiments describe the scheme of separation followed and summarize the analytical results obtained.

A 10-g. sample of the ground green Santos coffee was placed in a 75-ml. centrifuge tube. To this was added 50 ml. of distilled water at 25°C., a stopper was inserted, and the contents were shaken vigorously for a few minutes. After centrifuging, the supernatant liquid was decanted. The residue in the centrifuge tube was treated similarly with 3 additional 50-ml. portions of water (total of 200 ml.). Before extraction, the water was saturated with toluene to prevent bacterial decomposition. The combined extracts were filtered on suction and diluted to a volume of 200 ml. Portions of this extract were analyzed for total nitrogen, amino nitrogen, and caffeine nitrogen. The total nitrogen was determined by the Kjeldahl method; amino nitrogen was determined by means of a micro Van Slyke apparatus; all caffeine determinations were made at the Nestle Company Laboratories in Marysville, Ohio. Although trigonelline was not determined in these fractionation studies the original coffee contained 1.0%.

A 67 ml. aliquot of the aqueous extract was treated with 22 ml. of 15% trichloracetic acid solution and allowed to stand for 2 hours. The material was then filtered through quantitative filter paper and the precipitate washed with two 10-ml. portions of 2.5% trichloracetic acid solution. The combined filtrates were diluted to a volume of 100 ml. and analyzed for total, caffeine, and amino nitrogen; the precipitate was analyzed for total nitrogen.

Another 67-ml. aliquot of the extract was treated with 2 ml. of sulfuric acid, and then with 20 ml. of 20% phosphotungstic acid solution (made by dissolving 20 g. of phosphotungstic acid in a solution of 100 ml. of water to which was added 3 ml. of sulfuric acid). This reagent will precipitate proteins, proteoses, peptones, alkaloids, amines, and the basic amino acids; it should precipitate nearly all nitrogen compounds which would be expected to be present except simple peptides and the neutral and acidic amino acids. After standing 24 hours, the extract was filtered through quanti-

tative paper. The precipitate was washed with two 10-ml. portions of 2.5% phosphotungstic acid solution. Again, the combined filtrates were analyzed for total, caffeine, and amino nitrogen, and the total nitrogen of the precipitate was determined.

To secure a general idea regarding the combined amino acids which were present, samples of the green coffee, of the water extract, and of the precipitate and filtrate from the trichloracetic acid treatment of the water extract were hydrolyzed by boiling for 12 hours with 6 *N*. hydrochloric acid. The excess acid was removed from these hydrolyzates by distillation in vacuo, and the amino nitrogen content of the hydrolyzates was then determined.

A similar fractionation of the green coffee was carried out using 1% sodium hydroxide solution rather than distilled water as the extracting medium. Total nitrogen content of the various fractions was determined by the Kjeldahl method. Results, on the basis of the dry green bean, are reported in Table 3.

A portion of the Santos green coffee beans, from the same source as that used previously, was subjected to a medium roast. There was approximately 17% roasting loss. These roasted coffee beans were ground on a Wiley mill to pass a 20-mesh screen. This coffee was then fractionated according to the same scheme used for the green coffee. Results of this fractionation, reported on the basis of the dry roasted beans, are summarized in Table 4.

TABLE 3

Fractionation of soluble nitrogen compounds in green coffee

Sample	Total *N.*	Caffeine *N.*	Amino *N.*	Amino *N.* after hydrolysis
	%	%	%	%
Green Santos	2.28	0.32	.....	1.47
Water extract	1.28	0.32	0.16	0.81
TCA[b] ppt. from water extract	0.51	.....	.....	0.43
Filtrate from TCA ppt.	0.77	0.30	0.10	0.37
PTA[b] ppt. from water extract	1.21	.....	.....	.....
Filtrate from PTA ppt.	0.07	0.00	0.09	.....
1% NaOH extract	1.53	.....	.....	.....
TCA ppt. from alkaline extract	0.77	.....	.....	.....
Filtrate from TCA ppt.	0.75	.....	.....	.....
PTA ppt. from alkaline extract	1.43	.....	.....	.....
Filtrate from PTA ppt.	0.11	.....	.....	.....

[b]TCA indicates trichloracetic acid; PTA indicates phosphotungstic acid.

TABLE 4

Fractionation of soluble nitrogen compounds in roasted coffee

Sample	Total *N.*	Caffeine *N.*	Amino *N.*	Amino *N.* after hydrolysis
	%	%	%	%
Roasted Santos	2.37	0.33	.....	1.38
Water extract	0.59	0.34	0.08	0.20
TCA ppt. from water extract	0.01	.....	.....	0.01
Filtrate from TCA ppt.	0.58	0.31	0.09	0.19
PTA ppt. from water extract	0.50	.....	.....	.....
Filtrate from PTA ppt.	0.09	0.01	0.08	.....
1% NaOH extract	0.93	.....	.....	.....
TCA ppt. from alkaline extract	0.26	.....	.....	.....
Filtrate from TCA ppt.	0.67	.....	.....	.....
PTA ppt. from alkaline extract	0.80	.....	.....	.....
Filtrate from PTA ppt.	0.12	.....	.....	.....

DISCUSSION OF RESULTS

Results reported in Table 1 indicate that most of the protein which could be extracted from green coffee beans was water-soluble. An addi-

tional amount, however, was soluble in dilute alkali, and there was more alkali-soluble protein in Santos, a Brazilian coffee, than there was in Colombian, a Mild coffee. Further work on more samples would be necessary to determine if this is actually characteristic of these two coffee varieties. There was no appreciable globulin fraction (insoluble in water but soluble in dilute neutral salt solutions) and no prolamines (soluble in 80% ethanol). It should be noted, however, that a portion of the protein classed as water-soluble may actually belong to a globulin fraction. It is possible that some of this protein would be insoluble in pure water but was made soluble in the dilute salt solution formed by the soluble inorganic salts present in the coffee.

Nitrogen determinations on samples of water-soluble proteins prepared from the Santos and Colombian coffees indicated that the samples represented fairly pure protein preparations and that there was no significant difference in nitrogen content of these proteins from the two coffees. Furthermore, the water-soluble proteins from both coffees were found to have the same isoelectric point. Data reported in Table 2 indicated that there was no active protease present in the green coffee extracts, that the breakdown of proteins in the extracts was probably due to bacterial action, and that the proteins in the extract were stable for several days in the presence of a suitable preservative.

It was found (Table 3) that 56% of the total nitrogen in the green Santos beans was soluble in water; all the caffeine was water-soluble. When the extraction was made with 1% sodium hydroxide rather than water, 67% of the total nitrogen was present in the extract. The extra nitrogen, which was soluble in dilute alkali but not in water, was found to be protein in nature, since it was precipitated by trichloracetic acid.

Several of the fractions were hydrolyzed with hydrochloric acid; in all cases, this caused a large increase in amino nitrogen values. This was also true with the filtrate obtained after treatment of the water extract with trichloracetic acid, that is, after proteins were removed. This indicated that, in addition to proteins, there were considerable amounts of other amino acid-containing compounds present in the extract. These may have been present in the form of proteoses, peptones, peptides, and possible compounds related to Schiff bases.

Only 25% of the total nitrogen in the roasted Santos beans was soluble in water; again, all of the caffeine was soluble. There was a negligible amount of precipitate obtained by treating the aqueous extract of the roasted coffee with trichloracetic acid; apparently the water-soluble proteins of the green coffee were denatured and rendered insoluble in the roasting process. As with the green coffee, acid hydrolysis of the various fractions caused marked increases in amino nitrogen values. However, less of these intermediate products, soluble in trichloracetic acid solution but insoluble in phosphotungstic acid solution, were found in the roasted coffee extract than in the corresponding green coffee extract.

An alkaline extract of the roasted beans was found to contain 30% of the total nitrogen. The nitrogen compounds which were soluble in alkali but not in water were mainly protein. In all the extracts of both green and roasted coffee, phosphotungstic acid precipitated nearly all of the nitrogen present; the small amount not precipitated was accounted for as

free amino nitrogen. This may be in the form of amino acid-containing compounds of low molecular weight.

These survey studies have indicated certain areas where further work may be expected to prove profitable. It is possible that the extra alkali-soluble protein of the Brazilian coffee over that found in the Mild coffee may be of significance in explaining the difference in the roasted beans prepared from these coffees. This could only be established on study of a larger number of samples than is reported here. It is also suggested that the water-soluble amine or amino acid-containing compounds of the roasted coffee bean should be further investigated. There is an appreciable amount of this fraction present and it is apparently not in the form of proteins. It should be established whether this fraction represents protein decomposition products such as proteoses, peptones, and peptides, or polymerized materials of the Schiff base type. It might also be useful to learn whether the compounds are present as such in the green bean or are produced during the roasting process. Another fraction which should be further studied is that small group of nitrogen compounds present in the water extract of the roasted coffee, which are not precipitated by phosphotungstic acid. These appear to be comparatively simple primary amino compounds of some sort; they are apparently not strongly basic since they are soluble in phosphotungstic acid solution.

SUMMARY

This investigation has been primarily concerned with the protein fraction of the coffee bean. Approximately 3% of the green coffee was found to be present as water-soluble protein; an additional 1-2% protein was extracted with dilute alkali. The water-soluble protein was isolated from the green coffee beans and found to have a nitrogen content of 15.4%; the isoelectric point was at pH 4.6-4.7. No proteolytic enzymes active at pH 7.0 were found in aqueous extracts of the green coffee.

The nitrogen compounds of the green and roasted coffee were divided into fractions on the basis of solubility; water and dilute alkali were used as the extracting solvents and the extracts were further fractioned by use of trichloracetic acid and phosphotungstic acid. There were apparently appreciable amounts of non-protein amino acid-containing compounds present in water extracts of both green and roasted beans.

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A STUDY OF THE AMINO ACIDS OF GREEN AND ROASTED COFFEE INCLUDING A NEW METHOD OF PROTEIN HYDROLYSIS*

G. E. UNDERWOOD AND F. E. DEATHERAGE

Department of Agricultural Biochemistry, Institute of Nutrition and Food Technology, The Ohio State University, Columbus, Ohio

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Previous studies in this laboratory *(9)* had indicated that a large proportion of the nitrogen in the coffee seed or "bean" was present as amino acids, combined in the form of proteins or other compounds. It was believed that a study of the amino acids present in the raw and roasted beans might shed some light on the changes that take place in the roasting process. This work was done on a sample of Santos coffee.

EXPERIMENTAL

In order to learn which amino acids were present in the coffee, a 1-g. sample of the green Santos beans, ground in a hammer mill to approximately 40-mesh size, was mixed with 20 ml. of 6 *N*. hydrochloric acid and heated under reflux on an oil bath at 135°C. for 12 hours. The mixture was filtered on suction and the insoluble material washed several times with hot water. The excess acid was removed from the combined extracts by repeated distillation to dryness in vacuo. The residue was dissolved in water, neutralized, and diluted to a volume of 25 ml. Various concentrations of this hydrolyzate were then chromatographed on sheets of Whatman No. 1 filter paper, using a technique similar to that suggested by Consden, Gordon, and Martin *(3)*.

By this method it was possible to find the following amino acids in the green coffee hydrolyzate: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, leucine, lysine, phenylalanine, serine, threonine, tyrosine, and valine. Tryptophan was found to be present in the non-hydrolyzed coffee by use of the Adamkiewicz test.

Attention was next turned to a quantitative study of the amino acids present. The method used for separation of the amino acid mixtures produced by hydrolysis of various fractions was ion exchange chromatography. The technique was patterned after that of Moore and Stein *(4)*; the resin used was Dowex 50 *(1)*, a sulfonated polystyrene resin.

In this study a 120-cm. × 1.5-cm. Pyrex column was filled to a depth of 110 cm. with the sodium form of 200- to 400-mesh Dowex 50. The resin was obtained from the Dow Chemical Company; it was converted from the hydrogen form, as received, to the sodium form by treating with successive portions of 0.2 *N*. sodium hydroxide. The excess alkali and the resin "fines" were removed by repeated washing with distilled water; after thorough stirring, the resin was allowed to settle for a few minutes and the cloudy supernatant liquid was decanted. The resin was added to the column in the form of a thick slurry. The preparation of the column was completed by passing through 150 ml. of pH 3.5 buffer under a pressure of 200 mm. of mercury. The dry weight of the resin required to fill the column was 96 g.

The chromatographic operation was carried out by adding the amino acid mixture to the column and successively eluting with buffers of increasing pH. The buffers used were prepared from sodium acetate and hydrochloric acid, and all were 0.2 *N*. with respect to sodium. Preliminary experiments indicated that the order of emergence of the neutral and acidic amino acids found in the green coffee hydrolyzate was as reported in Table 1. This order was established by collecting small fractions of the effluent and determining the amino acids present in the various fractions by means of paper chromatography.

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TABLE 1

Order of emergence of amino acids from Dowex 50 column

pH 3.5	pH 4.5	pH 5.2
Aspartic acid	Leucine	Tyrosine
Threonine		Phenylalanine
Serine		
Glutamic acid		
Glycine		
Alanine		
Valine		

To test the quantitative applicability of the method, 1 mg. of each of aspartic acid, threonine, and serine, and 2 mg. of glutamic acid were dissolved in 5 ml. of water and added to the column. The acids were eluted from the column by adding the pH 3.5 buffer under a pressure of 200 mm. of mercury, and collecting approximately 2 ml. fractions of the effluent. A large sheet of filter paper was sprayed with 0.1% aqueous solution of ninhydrin and allowed to dry in air. Then small drops from each fraction were placed in consecutive order on this paper, by means of a small wire loop. After drying overnight at room temperature, the characteristic blue color had appeared in those spots which contained amino acids. By examination of the spots, it was possible to identify 4 definite peaks corresponding to the maximum concentration of the 4 amino acids. On the basis of these spots, the collected fractions were combined into 4 separate portions, representing the different amino acids.

In order to measure the percentage recovery of the various amino acids, use was made of the reaction of ninhydrin with the amino acid solutions. The procedure was similar to that described by Moore and Stein *(4)*. A standard leucine curve was constructed and, using this curve as a basis, the fractions collected from the ion exchange column were analyzed to determine the percentage recovery of the 4 amino acids (Table 2).

TABLE 2

Recovery of four amino acids on a Dowex 50 column

Amino acid	On column	Recovered	Recovery
	mg.	*mg.*	%
Aspartic acid	1.00	1.05	105
Threonine	1.00	0.95	95
Serine	1.00	1.03	103
Glutamic acid	2.00	1.99	100

In a subsequent run, a 2-mg. sample of leucine was added to the column and recovered in 97% yield. With these indications that the method gave nearly quantitative recovery of known amino acid mixtures, attention was focused on the analysis of the various hydrolyzates. The procedures used in preparing these hydrolyzates follows:

Green coffee. A 10-g. sample of the green, ground Santos coffee was mixed with 200 ml. of 6 *N*. hydrochloric acid and refluxed on an oil bath at 135°C. for 12 hours. The hydrolyzate was filtered on suction, the residue washed several times with hot water, and the excess acid removed from the combined filtrates by repeated distillation in vacuo. The residue was taken up in water, neutralized with sodium carbonate, diluted to a volume of 250 ml., and stored under toluene in the refrigerator.

Roasted coffee. A portion of the green Santos coffee beans was subjected to a medium roast; there was approximately 17% roasting loss. These roasted coffee beans were ground on a Wiley mill to pass a 20-mesh screen. A hydrolyzate was prepared from 10 g. of these roasted beans using the same conditions as described for the green coffee.

Water extract of green coffee. Two 10-g. samples of the green coffee were placed in separate 75-ml. centrifuge tubes. To each of these samples was added 50 ml. of distilled water at 25°C., a stopper was inserted, and the contents were shaken vigorously

for a few minutes. After centrifuging, the supernatant liquid was decanted. The residue in each centrifuge tube was treated similarly with 3 additional 50-ml. portions of water. Before extraction, the water was saturated with toluene to prevent bacterial action. The combined extracts were filtered on suction, evaporated to a volume of 100 ml. on the steam bath, and then treated with 100 ml. of concentrated hydrochloric acid. The mixture was refluxed for 12 hours, filtered, the excess acid removed, and the neutralized residue made up to a volume of 250 ml.

Water extract of roasted coffee. Six 10-g. samples of the roasted Santos coffee were extracted with water at 25°C., the combined extracts evaporated to a volume of 100 ml., and an acid hydrolyzate prepared as described for the water extract of the green coffee.

As noted previously, the Dowex 50 column is not suitable for analysis of the basic amino acids. To secure some information regarding the amount of these present in the hydrolyzates, they were determined as a group. This was done by treating a separate aliquot of the hydrolyzate with phosphotungstic acid. In addition to the basic amino acids, it is also a precipitant for alkaloids, such as caffeine and trigonelline, and for ammonia and some amines. In view of the report of Moores and Greninger *(5)* phosphotungstic acid may not completely precipitate trigonelline particularly from extracts of roasted coffee. If this is the case some of the non-basic nitrogen (Table 3) may be from trigonelline. Kjeldahl determinations were carried out on the phosphotungstic acid precipitate (basic nitrogen), on the filtrate (non-basic nitrogen), and on a separate portion of the hydrolyzate (total nitrogen). Results of these determinations on the different hydrolyzates, reported in terms of milligrams of nitrogen per gram of the dry green and roasted coffee bean, respectively, are summarized in Table 3. The

TABLE 3

Nitrogen content of coffee hydrolyzates

Sample	Nitrogen per gram of dry bean		
	Total	Basic	Non-basic
	mg.	*mg.*	*mg.*
Green Santos	21.8	10.7	11.0
Roasted Santos	19.8	9.6	9.9
Water extract of green Santos	12.5	7.0	5.5
Water extract of roasted Santos	5.7	4.8	0.94

moisture content of both green and roasted coffee samples was determined by drying for 2 hours in a vacuum oven at a temperature of 100°C. and a pressure of 4 inches of mercury.

A separate 5-ml. portion of each of the 4 hydrolyzates was analyzed for the neutral and acidic amino acids by use of the ion exchange column, using the technique previously described. The chromatographic operation was carried out under a pressure of 200 mm. of mercury and the flow rate through the column was about 47 ml. per hour. The hydrolyzates were highly colored and some of this color passed through the column. However, by the time the first amino acid appeared, the effluent liquid was water-clear and colorless so that the color of the hydrolyzate caused no difficulty in colorimetric determinations of the amino acids. The amount of the amino acids in the various hydrolyzates, calculated as milligrams of nitrogen per gram of the dry green or roasted coffee bean, respectively, are reported in Table 4.

Only very faint positive tests were found in the region where tyrosine should appear. Apparently only a small amount of this amino acid was present and it was contained in such a large volume of liquid that analysis for it by this method was not practical. In each instance, the amino acids determined accounted for approximately 80% of the non-basic nitrogen present in the hydrolyzates.

In the course of these investigations, the limitations of current methods of protein hydrolysis were noted. Thus, hydrolysis with strong acids results in the complete destruction of tryptophan and the partial decomposition of some of the other amino acids; in addition, large amounts of humin are

TABLE 4

Comparative amino acid content of coffee hydrolyzates

Amino acid	Nitrogen per gram of dry bean			
	Green bean	Water extract	Roasted bean	Water extract
	mg.	*mg.*	*mg.*	*mg.*
Alanine	0.74	0.36	0.72	0.03
Aspartic acid	1.35	0.70	1.20	0.14
Glutamic acid	2.32	1.18	2.00	0.27
Glycine	1.23	0.59	1.09	0.18
Leucine	1.43	0.69	1.26	0.10
Phenylalanine	0.52	0.26	0.46	0.02
Serine	0.42	0.22	0.36	0
Threonine	0.27	0.14	0.22	0
Valine	0.62	0.28	0.56	0.03
Total	8.90	4.42	7.87	0.77

formed. The use of strong alkalies causes racemization of the amino acids; there is some deamination; arginine is converted to ornithine and urea; and cystine is destroyed. Enzyme hydrolysis is unsatisfactory because of the long time required to secure complete hydrolysis and because of the fact that the enzymes are themselves proteins; the hydrolyzate may thus be contaminated by products split from the enzymes. In view of these facts, the need for improved methods of protein hydrolysis is apparent.

Steinhardt *(6)* reported that it was possible to secure some hydrolysis of proteins by use of certain high molecular weight sulfonic acids. It has also been observed *(7, 8)* that insoluble cation exchange resin in the hydrogen form act as catalysts for certain hydrolytic reactions, such as ester hydrolysis and sucrose inversion. However, apparently nothing has been reported with regard to the use of ion exchange resin for the hydrolysis of proteins. Since these materials had been shown to be effective catalysts in other hydrolysis reactions, it was decided to study their action on proteins.

The resin used in these experiments was Dowex 50, which behaves as an insoluble strong acid in aqueous solutions. The resin as received was in the hydrogen form and could be used directly after washing several times with water. An aqueous extract of this washed resin was found to have a pH of 4.2. After refluxing the resin 48 hours with 20 times its weight of water, the solution showed a faint positive test for sulfate; the pH of the solution was 4.0. The resin contained considerable moisture and the weights of resin used are reported in terms of this damp material; drying for 24 hours at 105°C. indicated a moisture content of 46%. The protein used for exploratory experiments was a commercial sample of vitamin-free casein, obtained from General Biochemicals, Inc., which was found to contain 14.87% nitrogen on a moisture-free, ash-free basis.

In a 250-ml. round-bottom flask were placed 1 g. of casein, 5 g. of 200- to 400-mesh Dowex 50, and 100 ml. of distilled water. This mixture was shaken vigorously, then refluxed on an oil bath which was left at a temperature of 135°C. Both the resin and the protein were insoluble and collected at the bottom of the flask; no stirring was used. By means of a pipet, samples of the mixture were withdrawn occasionally and the biuret test applied. Results are shown in Table 5.

In order to place the results on a quantitative basis, a hydrolysis was carried out similar to that described above except that no material was removed for biuret tests. After refluxing for 84 hours, the solution was filtered from the resin; this solution had a pH of 2.8. The resin was then extracted with three 40-ml. portions of 6 *N*. hydro-

TABLE 5

Rate of casein hydrolysis with Dowex 50

Reflux time	Biuret reaction
hours	
10	+
22	+
46	+
70	−

chloric acid to remove adsorbed hydrolytic products. Kjeldahl determinations on the combined extracts and on the resin indicated that the extraction of the hydrolysis products from the resin was about 98% complete. The neutralized extract was analyzed for its amino nitrogen content by the Van Slyke method *(10)*. Other runs were made in which the experimental conditions were varied, in order to find the optimum conditions for carrying out the hydrolysis. Thus, the effects of increasing the reflux time, of stirring the mixture, and of doubling the amount of resin were studied.

These amino nitrogen values were compared with the value obtained by hydrolyzing a sample of the casein with hydrochloric acid. A 1-g. sample of casein was hydrolyzed with 20 ml. of 6 *N*. hydrochloric acid by heating at 135°C. for 24 hours. In all cases, the resin hydrolyzates were clear and nearly colorless; in contrast, the hydrolyzate obtained by use of hydrochloric acid was very dark and contained considerable insoluble humin. The HCl hydrolyzate was neutralized and its amino nitrogen content determined. Results of these determinations are summarized in Table 6.

TABLE 6

Extent of casein hydrolysis with Dowex 50

Experimental conditions	Reflux time	Amino *N*.
	hours	%
5 parts resin	84	8.3
5 parts resin	132	8.6
5 parts resin	168	8.6
5 parts resin; mixture stirred	94	8.3
10 parts resin	90	8.9
6 *N*. hydrochloric acid	24	10.8

Since the use of an ion exchange resin for the hydrolysis of proteins had shown considerable promise in preliminary studies on casein, the method was extended to a study of the water-soluble proteins extracted from green Santos coffee. These proteins were secured by shaking 50 g. of the ground, green coffee beans with 400 ml. of distilled water, centrifuging, and filtering on suction. The filtrate was treated with 6 ml. of concentrated hydrochloric acid, and the precipitated protein was washed 5 times with 95% ethanol, then 5 times with ethyl ether. After drying 1 hour at 105°C., the nearly white solid was finely ground in a mortar, continuously extracted for 15 hours with absolute ether, then dried for 15 hours at 105°C. The nitrogen content of the product was 15.50%.

A hydrolyzate of the coffee protein was prepared by refluxing 400 mg. of protein with 8 ml. of 6 *N*. hydrochloric acid on an oil bath at 135°C. for 12 hours. After hydrolysis, the excess acid was removed by distillation in vacuo, the residue was taken up in water, neutralized, and diluted to a volume of 100 ml.

Another hydrolyzate was prepared by mixing 400 mg. of the coffee protein with 40 ml. of water and 2 g. of Dowex 50. The mixture was heated at reflux for 100 hours, the liquid was filtered from the resin and hydrolytic products were removed from the resin by washing the resin 3 times with saturated barium hydroxide solution. The excess barium was removed from the combined filtrates by use of sulfuric acid. The filtrate was finally adjusted to a volume of 100 ml. Again, the hydrolyzate obtained from the resin was nearly clear while that from the hydrochloric acid contained con-

siderable humin. To be certain that the low humin formation in the resin hydrolyzate was not due to the excess of liquid phase present, another portion of the protein was hydrolyzed for 12 hours with 200 times its weight of 6 *N*. hydrochloric acid. This procedure did appear to somewhat decrease humin formation, but the hydrolyzate still became very dark and some insoluble humin was produced.

A 5-ml. portion of each of the coffee protein hydrolyzates was chromatographed on the ion exchange column by the technique previously described, and the eluant fractions were analyzed for the various amino acids. Also, a 50-ml. portion of each hydrolyzate was treated with phosphotungstic acid to precipitate the basic constituents. Separate determinations of ammonia were made on 5-ml. portions of each hydrolyzate. This was done by making the solution basic with potassium carbonate and aerating for 1 hour into an excess of sulfuric acid. Titration of the excess acid with standard sodium hydroxide gave a measure of the ammonia present. The use of barium hydroxide for removing the amino acids from the resin was found to cause a large loss of ammonia. Therefore, the ammonia nitrogen of the resin hydrolyzate was determined by aerating a hydrolyzate in which the amino acids were removed from the resin by use of hydrochloric acid rather than barium hydroxide. Results of these analyses are reported in Table 7.

TABLE 7

Analysis of coffee protein hydrolyzates

	Nitrogen in	
	HCl hydrolyzate	Resin hydrolyzate
	mg.	*mg.*
Alanine	2.86	2.74
Aspartic acid	5.12	4.93
Glutamic acid	8.74	1.97
Glycine	4.53	4.38
Leucine	5.58	5.44
Phenylalanine	2.06	2.07
Serine	1.56	1.46
Threonine	0.95	0.94
Valine	2.57	2.39
Basic amino acids and humin	15.39	14.70
Ammonia	4.89	4.50
Total recovered	54.25	45.52
Total in hydrolyzate	61.60	61.61

By using the method of Graham, Smith, Hier, and Klein *(3)*, the tryptophan content of the coffee protein was found to be 1.7%, thus accounting for an additional 0.93 mg. of nitrogen in Table 7. Tryptophan is destroyed by acid hydrolysis or by refluxing with distilled water, hence was not found in either hydrolyzate.

DISCUSSION OF RESULTS

Data reported in Table 4 show that about one-half of the total amount of the amino acids determined in the green coffee beans was soluble in water. The corresponding ratio in the case of the roasted coffee was about 1:10. The amino acids analyzed for in the hydrolyzate of the green coffee accounted for approximately 8% of the total dry weight of the green coffee bean. The relative amounts of each of the amino acids in the green bean and the roasted bean were found to be comparable. In relation to the other amino acids, there was a definitely higher proportion of aspartic acid, glutamic acid, and glycine in the extract of the roasted coffee than there was in the roasted bean itself; the relative amount of leucine was about the same, while alanine, phenylalanine, and valine were distinctly

less in the extract. No serine or threonine was found in the roasted coffee extract. It may prove profitable to further investigate these water-soluble amino acid-containing compounds of the roasted coffee bean. There is an appreciable amount of this fraction present, representing about 3% of the total soluble solids, and it is apparently not in the form of proteins. This fraction may represent protein decomposition products such as proteoses, peptones, and peptides, or polymerized materials of the Schiff base type. It might also be useful to learn whether these compounds are present as such in the green bean or are produced during the roasting process.

It was found (Table 5) that it was possible to secure considerable hydrolysis of casein by merely mixing the protein with Dowex 50, adding water, and refluxing the mixture. In subsequent experiments (Table 6) it was shown that when the Dowex 50 was used, about 80% as much amino nitrogen was formed as was present in a hydrolyzate prepared by the classical method of hydrolyzing the protein with 6 *N*. hydrochloric acid. Doubling the amount of resin used or increasing the reflux time past 100 hours did not appreciably increase the amount of hydrolysis.

In analyses of two hydrolyzates prepared from water-soluble coffee proteins (Table 7), all of the amino acids determined were found to be present in the two hydrolyzates in comparable amounts with the exception of glutamic acid; there was much less of this acid in the hydrolyzate prepared using Dowex 50 than in the hydrochloric acid hydrolyzate. It is probable that the glutamic acid in the resin hydrolyzate formed pyrrolidone carboxylic acid *(11)*. The prolonged boiling and slightly acid medium would provide the ideal conditions for loss of water from glutamic acid, leading to formation of the pyrrolidone carboxylic acid. This reaction could also at least partly account for the low amino nitrogen values in the resin hydrolyzates of casein as reported in Table 6. Casein contains a high percentage of glutamic acid but the amino nitrogen is no longer free in pyrrolidone carboxylic acid. It was found that upon brief digestion with hydrochloric acid, glutamic acid appeared in the resin hydrolyzates in an amount comparable to that found in the HCl hydrolyzates.

Slightly more ammonia was found in the acid hydrolyzate of the coffee protein (Table 7), possibly indicating some decomposition which did not occur with the resin. Recent studies in this laboratory have indicated that there may be some simple peptides present in the resin hydrolyzates; however, the method appears to give nearly complete hydrolysis of the proteins studied.

Since existing methods of protein hydrolysis do have definite limitation, any new method deserves careful consideration. Areas for further research involving the use of ion exchange resins in the hydrolysis of proteins include a study of the effects obtained when different resins and different proteins are used; results obtained when strong acids and alkalies are used in conjunction with the resins; and the effect of conducting the resin hydrolysis at various temperatures and pressures. Since the hydrolytic conditions when the resin is used are quite mild and the hydrolysis proceeds slowly, it might be profitable to stop the hydrolysis after varying time intervals and study the products which have been produced. Such studies may provide information regarding the kinetics of protein

hydrolysis and perhaps be useful in establishing the original structure of the protein molecule. Such information regarding the effect of structure might lead to a more complete understanding of the steps involved in the action of enzymes. In addition, when the resin structures which give most rapid hydrolysis have been determined, it may be possible to construct a resin which will give very rapid and complete hydrolysis at a low temperature.

SUMMARY

In all, 14 amino acids were identified in hydrolyzates of green Santos coffee beans. By use of ion exchange chromatography, 9 of these amino acids were estimated quantitatively in hydrolyzates prepared from the green and roasted coffee and in hydrolyzates prepared from water extracts of raw and roasted coffee beans. All the amino acids determined were found to be present in the green and roasted beans in comparable amounts. There was an appreciable amount of amino acid-containing compounds present in the water extract of the roasted coffee.

A new method of protein hydrolysis, using an ion exchange resin, was found to give almost complete hydrolysis of casein and of the water-soluble coffee proteins. The resin used was Dowex 50, a sulfonated polystyrene resin; the hydrolysis was accomplished by mixing the protein, water, and the hydrogen form of the resin, and then refluxing the mixture.

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AMOUNT OF ASCORBIC ACID REQUIRED FOR HOME FREEZING OF ELBERTA PEACHES

ELSIE H. DAWSON, MARY E. KIRKPATRICK, AND EDWARD W. TOEPFER

Bureau of Human Nutrition and Home Economics, Agricultural Research Administration, U. S. Department of Agriculture, Washington, D. C.

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Browning and undesirable flavor changes occurring during freezing, storage, and thawing of frozen peaches are delayed by the addition of ascorbic acid, one of the most effective anti-darkening agents. Various quantities of ascorbic acid from 100 to 350 mg. per pint of fruit, have been recommended by different authors, depending on the conditions of freezing and the variety of peaches used *(1, 2, 3, 4, 5, 8, 10, 13, 14)*. The Elberta peach which was used in this study is a popular variety for freezing but has a tendency to brown rapidly when exposed to air *(4, 11, 15)*.

Using ascorbic acid in unnecessarily large quantities will increase unduly the cost of the frozen peaches. For this reason, experiments were undertaken to determine the minimum quantity of ascorbic acid required to maintain satisfactory color and flavor during the freezing and frozen storage of Elberta peaches under home conditions and to compare the relative requirements of dry sugar and of sirup packs.

PROCEDURE

Tree-ripened Elberta peaches, obtained from the orchards of the Bureau of Plant Industry, Soils and Agricultural Engineering of the United States Department of Agriculture, were peeled without scalding, using stainless steel knives, and sliced into twelfths or sixteenths depending on size. They were packed in 40% sugar sirup or with 1 part dry sugar to 4 parts fruit by weight. Crystalline L-ascorbic acid was added at various levels ranging from 33 mg. to 500 mg. per pint pack. Controls were prepared without added ascorbic acid. Three replications of all experimental samples were made.

For all samples 340 g. of sliced peaches were used, packed either with 191 g. of sirup or 85 g. of sugar. Ascorbic acid in amounts of 0, 33, 66, 132, 250 (sirup pack only), or 500 mg. per pint pack was dissolved in a small amount (25 ml.) of cold water. For the sirup packs, the dissolved ascorbic acid was mixed with the sirup before it was poured over the peaches. The amount of water used to dissolve the ascorbic acid was subtracted from the total amount needed to make a 40% sirup. For the sugar packs, the dissolved ascorbic acid was added to the peaches and carefully distributed over the slices for 30 seconds with a rubber spatula. Sugar was then added and peaches were carefully mixed 4 times for 30 seconds each at 5-minute intervals, to aid in formation of sirup and even distribution of ascorbic acid, sugar, and peaches.

All samples were packaged in moisture-vapor-resistant cellophane-lined paperboard cartons with a piece of crumpled parchment paper in the top to keep the fruit immersed in the sirup. Air was pressed out of the bags and they were heat sealed.

The samples were frozen at 0°F.(−18°C.) in a freezer cabinet with eutectic plates on 3 sides and a fan to circulate the air. Cartons were pyramided along the 3 sides, with approximately ¼ inch space between them. Samples were stored at 0°F.(−18°C.) or at −40°F.(−40°C.) in still air.

The delayed method of testing *(9)* in which all storage samples are examined simultaneously was employed for all packs containing 0 to 132 mg. of ascorbic acid.

Those intended for testing after 10 months at 0°F.(−18°C.) were placed immediately in storage at this temperature. The remaining material was held at −40°F.(−40°C.) and after 6 months the 4-month experimental samples were transferred to 0°F.(−18°C.) storage. Representative samples were held for 10 months at −40°F.(−40°C.). Because peaches stored at this sub-zero temperature retained the color and flavor of the freshly frozen fruit, they served as excellent controls for the peaches stored at the higher experimental temperature and justified the use of the delayed method of testing for frozen peaches.

The packs containing 250 and 500 mg. of ascorbic acid, prepared the previous season, were stored at 0°F.(−18°C.), and sampled at intervals of 0, 4, and 10 months of storage.

In preparation for palatability tests the peaches were thawed in the carton on a wire rack in a room held at 75°F.(24°C.). Immediately after thawing, random samples of the peaches were served on small white china plates to 6 trained judges who were seated at small individual tables, each equipped with a special Macbeth daylight lamp. Only 4 samples were tested at any one tasting session. A 5-point numerical scale with 5 as the most desirable and 1 as the least desirable score was used to rate color, flavor, and acceptability.

Ascorbic acid content was determined as reduced ascorbic acid, using either the titrimetric procedure *(12)*, with corrections for reductones if present, by the method of Mapson *(6)*, or the photometric procedure *(7)* on raw and freshly processed samples and on those stored for 10 months.

RESULTS AND DISCUSSION

Palatability comparisons showed that when frozen without ascorbic acid, peaches in the sirup packs were generally superior to those in the sugar packs. On a 5-point scale, the acceptability score for the freshly frozen peaches in the sirup pack without added ascorbic acid was 4.0 as compared with 3.3 for those packed in dry sugar, and after storage for 10 months at 0°F.(−18°C.) the scores were 3.5 and 2.9, respectively. These differences in scores for sugar and sirup packs were statistically significant at the 1% level. Both color and flavor of the peaches in the sirup packs were rated higher than in the sugar packs. The latter had considerable amounts of oxidized flavor. This confirms the findings of Sater, Kirkpatrick, Stein, Murray, and Skinner *(8)* who reported that sirup-packed peaches were superior to corresponding dry sugar packs, regardless of treatment.

With respect to sweetness, the panel rated the fruit as well as the sirup too sweet in the sugar packs. In no instance was the fruit rated too sweet in the sirup packs although in a few instances it was rated too sour.

The mean scores in Table 1 show the effect of various levels of added ascorbic acid upon the color of peaches in sirup and sugar packs as freshly processed and after storage. Ascorbic acid added at the rate of 33 to 66 mg. per pint package in the sirup pack appears adequate to maintain color scores above 4, or good, throughout storage for 10 months. Higher levels of ascorbic acid gave further improvement; 500-mg. additions gave excellent color retention throughout the 10-month storage period.

In this study, the color of the peaches was rated immediately after thawing, as it is recommended for home use that frozen fruit be served as soon as thawed *(2)*. This accounts for the small amount of ascorbic acid needed to maintain good color in home-frozen peaches packed in sirup. Strachan and Moyls *(9)* reported that for Elberta peaches 200 mg. of ascorbic acid appeared to be the minimum quantity necessary for reason-

TABLE 1

Color scores for frozen sliced Elberta peaches with various levels of added ascorbic acid

Type of pack	Ascorbic acid added per pint	Mean color scores[a]			
		Freshly processed	Stored at −40°F. for 10 months	Stored at 0°F. for	
				4 months	10 months
	mg.				
Sirup pack	0	3.5	4.0	3.8	3.9
	33	4.6	4.6	4.4	4.0
	66	4.8	4.5	4.7	4.6
	132	4.6	4.6	4.6	4.3
	250	4.9	....	4.9	4.6
	500	4.9	....	4.9	4.8
Sugar pack	0	2.5	2.8	3.2	3.1
	33	3.5	3.7	3.5	3.5
	66	4.1	4.2	4.0	3.5
	132	4.6	4.3	4.2	3.8
	500	4.9	....	4.9	4.6

[a]Average of three replicates scored by a panel of 6 persons. A score of 5 is the highest possible score, 1 the lowest.

ably beneficial effects in retaining color and flavor of thawed fruit for at least one hour of exposure to air.

In the sugar packs larger amounts of added ascorbic acid were required to maintain color scores equal to those of the sirup packs. Packs with 500 mg. of added ascorbic acid maintained color scores above 4 for 10 months at 0°F.(−18°C.). Although the initial scores for the sugar packs with 132 mg. of ascorbic acid were good, the color dropped below score 4 after storage for 10 months at 0°F.(−18°C.).

Flavor scores (Table 2) followed a trend similar to the color scores. At least 66 mg. of ascorbic acid was needed in the sirup packs to maintain flavor scores of 4 or above throughout storage for 10 months at 0°F. (−18°C.). In the sugar packs, the peaches with 500 mg. of added ascorbic

TABLE 2

Flavor scores for frozen sliced Elberta peaches with various levels of added ascorbic acid

Type of pack	Ascorbic acid added per pint	Mean flavor scores[b]			
		Freshly processed	Stored at −40°F. for 10 months	Stored at 0°F. for	
				4 months	10 months
	mg.				
Sirup pack	0	4.4	4.5	4.3	3.6
	33	4.6	4.6	4.5	3.6
	66	4.4	4.5	4.4	4.2
	132	4.5	4.8	4.4	4.1
	250	4.7	....	4.6	4.0
	500	4.7	....	4.7	4.2
Sugar pack	0	4.0	4.0	4.0	3.0
	33	4.4	4.3	4.4	3.1
	66	4.5	4.7	4.4	3.8
	132	4.4	4.6	4.5	3.7
	500	4.7	....	4.5	4.2

[b]Average of three replicates scored by a panel of 6 persons. A score of 5 is the highest possible score, 1 the lowest.

acid maintained flavor scores above 4 for 10 months. However, 500 mg. is probably more than would be needed to maintain good quality for 10 months. Since no sugar packs containing 250 mg. were prepared, the amount actually needed must be estimated. By interpolation it was found that approximately 225 mg. of ascorbic acid would be adequate in sugar packs to maintain flavor and color scores of 4 during storage for 10 months at 0°F.(—18°C.).

Analysis of variance also showed that both storage time and level of added ascorbic acid caused highly significant differences in color and flavor scores. Regression coefficients for both flavor and color scores of frozen peaches stored for 10 months at 0°F.(—18°C.) were negative, indicating deterioration during storage.

TABLE 3

Ascorbic acid content of fresh and frozen Elberta peaches with various levels of added ascorbic acid

Type of pack	Ascorbic acid added per pint	Ascorbic acid content					Difference required for significance at 5% level	Ascorbic acid retention	
		Fresh peaches plus sirup or sugar	Freshly frozen	After storage at °F. for				Fresh to freshly frozen	Fresh to frozen, stored 10 months
				4 months	10 months	12 months			
	mg.	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*		%	%
Sirup pack	0	3.5	3.2	3.8	3.9	.....	0.7	91	111
	33	9.7	5.0	5.3	5.5	.....	0.9	52	57
	66	15.9	10.4	10.0	9.4	.....	1.4	65	59
	132	28.4	19.4	23.1	18.7	.....	2.6	68	66
	250	.....	43.6	48.1	44.6	37.7	9.8	....	....
	500	.....	80.0	86.0	79.6	72.7	6.7	....	....
Sugar pack	0	4.1	4.0	3.9	3.2	.....	0.7	98	78
	33	11.4	5.6	5.1	4.5	.....	0.8	49	39
	66	18.8	8.1	9.1	8.5	.....	0.9	43	45
	132	33.4	16.0	17.2	16.5	.....	2.1	48	49
	500	.....	84.1	92.6	91.0	89.1	19.5	....	....

There were significant losses of reduced ascorbic acid during preparation and freezing in both sirup and sugar packs to which ascorbic acid had been added (Table 3). Retention or loss of total biologically active vitamin C may not be inferred since the data were based on reduced ascorbic acid values and do not include dehydroascorbic acid values. In the freshly frozen product, retention of reduced ascorbic acid in the sirup packs with 33 to 132 mg. of added ascorbic acid ranged from 52 to 68%, and in the sugar packs, from 43 to 49%. Somewhat higher retention figures in the sirup packs illustrate again the greater protection against oxidative changes conferred by sirup packs as compared with sugar packs. After freezing and during storage for 10 months at 0°F.(—18°C.) there were no significant changes in the ascorbic acid content of any of the packs. This observation, that after freezing there were no further losses of ascorbic acid, agreed with that reported by Strachan and Moyls *(10)* on the retention of ascorbic acid in frozen stored peaches. In packs with 150 and 250 mg. of ascorbic acid added to 426 g. of fruit and sirup, there were no increases

in the loss of ascorbic acid at the 16-month period over those observed at the 8-month period. They reported 59 to 72% retention of ascorbic acid in comparable packs.

SUMMARY

In both flavor and color scores, there was deterioration during storage as evidenced by negative regression coefficients from freshly frozen peaches to peaches stored for 10 months at 0°F.(−18°C.). This occurred in both the sirup and sugar packed fruit.

Larger quantities of ascorbic acid were needed to preserve quality during storage of frozen Elberta peaches in the sugar pack than in the sirup pack. For the sugar pack, it was estimated that approximately 225 mg. of ascorbic acid per pint package was required to maintain a palatability score of good. In the sirup pack, the minimum quantity required to maintain good quality in Elberta peaches stored at 0°F.(−18°C.) for 10 months was found to be 66 mg. per pint package.

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STUDIES ON THE CHEMICAL NATURE OF AN ANTIBIOTIC PRESENT IN WATER EXTRACTS OF CABBAGE[a]

J. M. DICKERMAN AND S. LIBERMAN
Massachusetts Agricultural Experiment Station, Department of Bacteriology, University of Massachusetts, Amherst, Massachusetts

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In a preliminary investigation, water extracts of various plants, such as beets, cabbages, carrots, corn, green beans, potatoes, and spinach were tested for antibacterial activity. By the methods employed, only the water extract of cabbage showed any appreciable bactericidal action. Therefore, the object of the present investigation was to attempt to isolate and identify chemically the antibiotic present in cabbage.

Sherman and Hodge *(7)* demonstrated the bactericidal action of water extracts of cabbages and turnips against *Escherichia coli*, *Aerobacter aerogenes*, *Pseudomonas campestre*, and the natural mixed flora from the exterior of these plants. The action of the bactericidal substance was destroyed after an exposure period of 10 minutes at 60°C., and was removed by passage through a Berkefeld N filter.

Pederson and Fisher *(6)* showed that the normal flora present upon the surface of cabbage leaves, which included species of the genera *Achromobacter*, *Flavobacterium*, and *Pseudomonas*, ordinarily disappeared shortly after the cabbage was cut. The antibacterial substance was extracted with water and was shown to be heat labile.

Little and Grubaugh *(3)* tested the bactericidal action of extracts of cabbage bred to resist "cabbage yellows," a disease caused by *Fusarium oxysporum f. conglutanins*. The extracts were found to be more active against animal than plant pathogens.

EXPERIMENTAL PROCEDURES

Freshly picked heads of cabbage were used in these experiments, since it was found that storage markedly decreased the activity of the antibiotic.

Preparation and concentration of the crude extract. The crude extract was prepared by homogenizing a 200-g. composite sample of several heads of Danish Ball Heal cabbage in a Waring blendor with 100 ml. of cold (10°C.) distilled water. The extract was then centrifugated at 2,000 r.p.m. for 10 minutes, sterilized by passage through a Seitz filter, and stored at 10°C. to prevent any loss of bactericidal activity. Approximately 200 ml. of crude cabbage extract, with a pH value of 6.8, was obtained by this procedure from 200 g. of cabbage. The antibacterial activity of 4 extracts (I-a, I-b, I-c, I-d), prepared in this manner, is shown in Table 1. The most recently prepared extract (I-d) was concentrated by the pervaporation method *(3)*. The results of concentrating 90 ml. of the crude extract to 30 ml. (I-d3) and to 10 ml. (I-d9), respectively, is shown in Table 1.

The paper-disc agar-plate method *(1, 8)* was used for the demonstration of antibacterial activity against: *Streptococcus pyogenes*, *Micrococcus pyogenes var. aureus p 209*, *Escherichia coli*, *Salmonella typhosa*, *Bacillus cereus var. mycoides*, *Bacillus subtilis*, and *Bacillus coagulans*. Readings of the diameter of the zone of inhibition were

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made after 16 hours of incubation at 37°C., with the exception of *B. coagulans* which required an incubation period of 24 hours.

Purification procedures. Preliminary studies showed that the bactericidal factor was dialyzable through a cellophane dialysis sack placed in cold (10°C.) distilled water. Therefore, dialysis of the crude concentrated extract (I-d9) served as the first step in the purification procedure. An attempt was then made to purify the dialysate by the use of the various chemical agents such as phosphotungstic acid, ammonium sulphate, trichloracetic acid, ether, acetone, ethyl alcohol, and secondary butanol, equilibrated with water. However, none of these agents, with the exception of ethanol, removed any appreciable amount of the antibacterial activity. Eight volumes of alcohol were required to precipitate the antibiotic substance, giving an extremely small yield of the purified material. Therefore, paper partition chromatography, which requires a very small amount of material, was used as a means of isolating and purifying the active agent.

TABLE 1

The antibacterial activity of sterile crude water extracts of cabbage

Organisms	Diameter of the zone of inhibition in mm.					
	Original crude extracts				Concentrated crude extracts	
	I-a	I-b	I-c	I-d	I-d3	I-d9
Strep. pyogenes	15	15	17	15	20	25
Mic. pyogenes var. aureus	19	15	18	15	27	31
E. coli	19	15	18	18	23	27
S. typhosa	28	25	23	23	35	36
B. cereus var. mycoides	20	18	21	21	29	30
B. subtilis	18	16	18	17	27	29
B. coagulans	17	19	18	17	21	23

A modification of the paper chromatographic technic *(4, 5)* was used with various solvent mixtures. Components possessing free amino groups or strongly reducing properties were detected by the use of the ninhydrin or the ammoniacal silver nitrate reagent. Separation of the active bactericidal factor from the dialysate was obtained with a solvent that consisted of pyridine, water, and ethyl acetate in the organic phase in a ratio of 25:35:75, respectively. In this case, the active bactericidal agent had an R_f value of 0.4 and reacted only with the ammoniacal silver nitrate, indicating a strongly reducing substance lacking free amino groups. Results similar to these were obtained with a solution of the alcohol-precipitated material.

Studies on the chemical nature of the antibiotic. Since carbohydrate substances may act as reducing agents, Molisch and Benedict's tests were performed on material eluted from various areas of the chromatogram. Positive tests were obtained only with material eluted from the area where the ammoniacal silver nitrate had given a positive reaction.

Spectrographic analysis of the dialysate and of material eluted from the portion of the chromatogram showing antibacterial activity did not indicate the presence of any metals that might have an oligodynamic action. Absorption spectrophotometric studies of the dialysate revealed the presence of a substance having the same absorptive band as ascorbic acid which is a strongly reducing substance. Experiments showed that a concentration of 0.6% or less of ascorbic acid had no appreciable antibacterial effect upon the various organisms previously mentioned, even when this amount was added to a crude concentrated extract that was devoid of antibacterial activity. This amount (0.6%) was far greater than that shown by chemical determination to exist in the original crude concentrated extract (I-d9). Likewise, a negative chemical test for ascorbic acid was obtained with the alcohol-purified material.

DISCUSSION

Studies on the chemical nature of the antibacterial agent indicated that it was completely dialyzable through a cellophane sack which does

not allow the passage of molecules with a gram-molecular weight greater than 10,000. This fact tends to rule out the presence of large protein molecules such as those comprising many of the enzymes found in nature. Further proof in favor of this assumption is the fact that none of the so-called protein precipitation agents, except ethanol, removed any of the antibacterial activity from the concentrated extract. Eight volumes of ethanol were required to remove any of the active principle. The addition of 6-8 volumes of ethanol is a common procedure for the removal of polysaccharides from aqueous solutions, whereas 2-4 volumes of ethanol usually suffice to remove proteinaceous substances.

Additional evidence, obtained by paper chromatography, indicated that the antibacterial agent was carbohydrate in nature. The active agent, when eluted from the chromatogram, showed strongly reducing properties as well as positive Molisch and Benedict's reactions. The eluted agent also showed a lack of free amino groups, which again tends to rule out the presence of proteinaceous material.

Spectrographic analysis of the dialysate and of the material eluted from the chromatogram did not indicate the presence of any metals that might possess oligodynamic properties. Results obtained with the dialysate, using an absorption spectrophotometer, indicated the presence of ascorbic acid which is a strongly reducing substance of a carbohydrate nature. However, no ascorbic acid was demonstrable in material eluted from the chromatogram as shown by the absorption spectrophotometer. A chemical analysis of the concentrated crude extract (I-d9) for ascorbic acid failed to reveal the presence of an amount great enough to cause the antibacterial activity of the dialysate. Likewise, a chemical analysis of the alcohol-purified material did not indicate the presence of any ascorbic acid. Therefore, it would appear that the antibacterial agent is of a carbohydrate nature with a gram-molecular weight of 10,000 or less. It also appears that the antibacterial agent is not ascorbic acid.

There is little information, at the present time, about the mode of action of the antibacterial agent. From the data presented (Table 1), it is evident that the antibacterial substance is active against a variety of gram-positive and gram-negative bacteria.

SUMMARY

An antibiotic, effective against a variety of gram-positive and gram-negative bacteria, was shown to exist in water extracts of freshly picked cabbage. Studies on the chemical nature of the antibacterial agent seem to indicate that it is a strongly reducing substance of a carbohydrate rather than of a protein nature, with a gram-molecular weight of 10,000 or less.

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THE ESSENTIAL AMINO ACID CONTENT OF SEVERAL VEGETABLES[a]

JUNIATA S. HIRSCH, ALICE D. NILES, AND A. R. KEMMERER
Department of Nutrition, University of Arizona, Tucson, Arizona

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Even though the general use of microbiological methods for the determination of amino acids has produced a considerable volume of published data on the amino acid content of many proteins, only limited data are available on the amino acid content of vegetables. Stokes, Gunness, Dwyer, and Caswell *(9)* give values for the essential amino acid content of carrots, peas, and potatoes. Lyman and Kuiken *(7)* report values for the essential amino acid content of several vegetables, and Chitre, Williams, and Elvehjem *(1)*, the essential amino acid content of raw and canned peas. This laboratory previously reported the essential amino acid content of broccoli, cauliflower, carrots, and sweet corn *(4)*.

In this paper are reported the essential amino acid contents of fresh and dried blackeye peas, broccoli heads and stalks, spinach, potatoes, sweet potatoes, pinto beans, and raw and roasted peanuts. These vegetables were grown in the southwestern desert area, and were obtained from local markets.

EXPERIMENTAL

Three different lots of fresh and dried blackeye peas, broccoli, spinach, potatoes, sweet potatoes, and pinto beans were obtained from retail markets. Lots were purchased from markets supplied by different wholesalers, or from the same market at different times, in an effort to obtain samples from different production lots. Three lots of raw Spanish peanuts were obtained directly from oil mills in New Mexico and Texas. The fresh vegetables were blanched in an autoclave, homogenized for 2 to 5 minutes in a Waring blendor with an equal weight of water, and stored in a deep freeze at 0° to 2°F.(−18° to −16.5°C.) until analyzed. The dried blackeye peas and pinto beans were ground in a hammer mill and stored at room temperature until analyzed. One-half of each lot of shelled, raw peanuts was roasted by heating for 40 minutes at 320°F.(160°C.) and both raw and roasted peanuts were chopped as finely as possible in a Waring blendor. A large portion of the oil in the peanuts was removed by homogenizing equal parts of chopped peanuts and Skelly Solve B in a Waring blendor followed by filtering in a Buchner funnel. This procedure was repeated two more times. The samples then were air-dried and stored at room temperature. This "oil-low" material was used for the assay of amino acids in peanuts.

For assay of the amino acids, 5- to 40-g. quantities of each lot of each vegetable were refluxed in triplicate with 20% HCl (constant boiling) for 24 hours and assayed microbiologically as previously reported *(4)*. This procedure is that of Henderson and Snell *(5)* with slight modification. *Streptococcus faecalis R* was used for the assay of arginine, methionine, threonine, and tryptophan. *Lactobacillus arabinosus 17-5* was used for the assay of leucine and valine. *Leuconostoc mesenteroides P-60* was used for histidine, isoleucine, lysine, and phenylalanine.

The L forms of arginine, histidine, and lysine were used in preparing standard curves for the amino acid assays. DL forms of all other amino acids were used. When both L and DL forms were available, the response of the assay organism to each was

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determined. Each amino acid used for standard curves was tested for contamination with each of the other 9 essential amino acids by microbiological assay. All of the crystalline amino acids used for the standard curves were dried at 194°F.(90°C.) and stored in a desiccator except histidine and lysine which contained water of crystallization. None of the amino acids used was found to contain more than 0.30% moisture even before drying. Finally the crystalline amino acids were checked against U.S.P. standard amino acids.

For the determination of the moisture content of the vegetables, a Brabender Semi-Automatic Moisture Tester was used. Protein (N × 6.25) was determined by a Micro-Kjeldahl procedure *(5)*. Oil determinations were made on both the original and "oil-low" peanut material by extracting the peanuts with Skelly Solve B for 16 hours in a Soxhlet-type extractor.

RESULTS AND DISCUSSION

The average percentages of moisture, of protein, and of essential amino acids of the 10 vegetables are given in Table 1. The amino acid content is calculated on the fresh weight, dry weight, and protein (N × 6.25) basis. On a dry weight and protein basis, fresh blackeye peas are similar to dried blackeye peas in amino acid content. Broccoli heads are the same or slightly higher than broccoli stalks in all amino acids except lysine. With the possible exception of arginine, raw and roasted peanuts differ only slightly in amino acid content indicating that the roasting process had little effect on the total amino acids. Sweet potatoes are higher than potatoes in many of the essential amino acids even though the total protein content of sweet potatoes is slightly lower. Values for broccoli are in general agreement with values reported previously from this laboratory by Kemmerer and Acosta *(4)*. Values for dried blackeye peas and potatoes are in good agreement with values for these vegetables reported by Lyman and Kuiken *(7)*, but amino acid values reported in the present paper for spinach and sweet potato are substantially higher than those reported by Lyman and Kuiken *(7)*.

Each value in Table 1 is an average of values for three lots of each vegetable. Since each lot was assayed 2 to 18 times, opportunity was afforded to determine, by statistical analysis, the variations in amino acid content of the protein fraction of each vegetable. Student's t test was used as a test of the significance of variations among lots. For all of the vegetables there was a significant lot variation in 1 to 9 of the amino acids. The least variation occurred among the three lots of dried blackeye peas, and the most variation occurred among the lots of spinach. Whether this variation was due to differences in lot or to other unrecognized factors cannot be proved. However, in a metabolizing plant, such as fresh spinach, free amino acids would occur, and could cause variations between lots. From the statistical analysis, it appears that for many foods, especially raw or green vegetables, more than one lot should be assayed for reliable results.

The standard error and the percentage error of the mean of all values for each vegetable also were calculated. The percentage errors of the means are listed in Table 1. The greatest errors for any one amino acid were found among the tryptophan values.

Mitchell and Block *(8)* and Dunn *(2)* used whole egg protein as a standard of nutritive excellence and compared the amino acid make-up

TABLE 1

Essential amino acid content of ten vegetables

Vegetable	Protein content (N×6.25) dry weight basis	Moisture content	Average amino acid content									
			Phenylalanine	Leucine	Valine	Arginine	Methionine	Threonine	Histidine	Lysine	Isoleucine	Tryptophan
	%	%	%	%	%	%	%	%	%	%	%	%
Fresh blackeye peas	28.2	74.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh weight			0.403	0.484	0.378	0.442	0.101	0.274	0.239	0.461	0.337	0.072
Dry weight			1.56	1.88	1.97	1.71	0.391	1.06	0.926	1.78	1.31	0.279
Protein			5.53	6.66	5.21	6.06	1.39	3.76	3.28	6.31	4.64	0.989
Percent error[b]			3.9	1.5	2.6	2.2	2.1	3.7	3.0	2.9	1.5	3.1
Dried blackeye peas	25.0	8.70	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh weight			1.17	1.76	1.31	1.45	0.289	0.897	0.719	1.44	1.09	0.229
Dry weight			1.28	1.93	1.43	1.59	0.316	0.982	0.788	1.58	1.19	0.251
Protein			5.12	7.72	5.72	6.36	1.26	3.93	3.15	6.32	4.76	1.00
Percent error			1.8	1.4	1.8	2.0	4.0	1.8	3.1	4.2	3.1	1.3
Broccoli heads	40.7	86.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh weight			0.220	0.261	0.309	0.357	0.067	0.202	0.112	0.148	0.216	0.067
Dry weight			1.64	1.95	2.31	2.66	0.500	1.51	0.836	1.10	1.61	0.500
Protein			4.03	4.80	5.68	6.54	1.23	3.71	2.05	2.70	3.96	1.23
Percent error			2.0	2.1	2.1	4.8	3.7	1.9	2.8	4.3	1.5	1.8
Broccoli stalks	23.6	91.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh weight			0.073	0.089	0.113	0.106	0.026	0.076	0.037	0.103	0.075	0.020
Dry weight			0.859	1.05	1.33	1.25	0.306	0.894	0.435	1.21	0.882	0.235
Protein			3.64	4.45	5.64	5.30	1.30	3.79	1.84	5.13	3.74	0.996
Percent error			1.6	1.4	3.9	4.9	1.2	2.6	2.5	2.3	3.0	11.5
Spinach	24.1	88.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh weight			0.149	0.222	0.197	0.190	0.055	0.152	0.077	0.209	0.148	0.053
Dry weight			1.30	1.93	1.71	1.65	0.478	1.32	0.670	1.82	1.29	0.461
Protein			5.39	8.01	7.10	6.35	1.98	5.48	2.78	7.55	5.35	1.91
Percent error			2.3	1.4	1.6	2.9	4.2	1.5	2.1	5.4	1.5	2.0

Potatoes	7.45	75.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh weight			0.088	0.090	0.108	0.093	0.027	0.072	0.027	0.101	0.079	0.019
Dry weight			0.353	0.361	0.434	0.373	0.108	0.289	0.108	0.406	0.317	0.076
Protein			4.74	4.84	5.82	5.01	1.45	3.88	1.45	5.45	4.26	0.102
Percent error			4.0	0.6	3.0	1.9	6.9	2.2	5.3	4.3	4.1	17.1
Sweet potatoes	6.32	74.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh weight			0.100	0.092	0.152	0.085	0.042	0.093	0.040	0.091	0.083	0.035
Dry weight			0.385	0.354	0.585	0.327	0.161	0.358	0.154	0.350	0.319	0.135
Protein			6.09	5.60	9.26	5.17	2.55	5.66	2.44	5.54	5.05	2.14
Percent error			2.9	1.9	1.7	3.0	1.2	1.9	2.0	2.0	1.6	15.0
Pinto beans	20.5	8.82	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh weight			1.15	1.54	1.17	1.01	0.183	0.810	0.543	1.28	1.06	0.183
Dry weight			1.26	1.69	1.28	1.11	0.201	0.888	0.596	1.40	1.16	0.201
Protein			6.15	8.24	6.24	5.41	0.98	4.33	2.91	6.83	5.66	0.980
Percent error			3.3	1.0	1.9	2.9	1.7	1.8	1.4	3.6	3.4	4.9
Raw peanuts	29.9	4.01	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh weight			1.59	1.43	1.56	3.04	0.255	0.839	0.814	1.12	1.30	0.325
Dry weight			1.66	1.49	1.63	3.17	0.266	0.874	0.848	1.17	1.35	0.338
Protein			5.55	4.98	5.45	10.60	0.890	2.92	2.84	3.91	4.52	1.13
Percent error			3.1	1.4	1.6	4.5	4.2	2.6	5.0	2.8	1.8	7.0
Roasted peanuts	30.0	0.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fresh weight			1.38	1.42	1.48	2.60	0.261	0.764	0.850	1.17	1.21	0.302
Dry weight			1.38	1.42	1.48	2.60	0.261	0.764	0.850	1.17	1.21	0.302
Protein			4.60	4.73	4.93	8.67	0.870	2.55	2.83	3.90	4.03	1.01
Percent error			2.5	2.5	2.0	3.0	3.5	3.5	2.2	2.6	2.5	4.2
Whole egg protein *(2)*	.....	.....	5.4	9.2	7.1	5.7	3.1	5.3	2.4	7.3	6.7	1.6

[b] Percent error (protein basis) = (standard error of mean × 100) / mean.

of other proteins with that of whole egg. Such comparisons may be made from Table 1 in which the essential amino acid content of whole egg protein and of the 10 vegetable proteins is given. Using whole egg protein as a criterion, potato protein is low in all the 10 essential amino acids. Broccoli heads and stalks are low in all but one amino acid, arginine. Roasted peanuts are low in 8 amino acids; fresh and dried blackeye peas and raw peanuts are low in 7 amino acids; and pinto beans are low in 6 amino acids. Sweet potato is low in arginine, isoleucine, leucine, and lysine. Spinach is low in isoleucine, leucine, and methionine. Considering whole egg as the criterion, isoleucine, leucine, and methionine are the amino acids which most often are limiting factors in the vegetables assayed.

The response of the assay organisms to the L and DL forms of 9 amino acids is given in Table 2. DL isomers of histidine, isoleucine, lysine, methionine, phenylalanine, threonine, and tryptophan were approximately 50% as active as their respective L isomers for the organisms used. The activity of DL leucine was 57% of that of the L isomer, and the activity of DL valine was 61% of that of the L isomer. Both the L and DL forms of these two amino acids were checked for contamination with the other essential amino acids by microbiological assay. Contamination of the L valine probably accounts for the apparent high activity of the DL isomer. Since samples of L and DL leucine free of contamination were used, it appears possible that DL leucine is slightly more than 50% as active as L leucine for *Lactobacillus arabinosus 17-5*. This is in agreement with Lewis, Snell, Hirschmann, and Fraenkel-Conrat *(6)* who report 58% activity for DL leucine. However, all of the amino acid values given in Table 1 are expressed on the basis of 100% activity for the L isomers and 50% activity for the DL isomers.

TABLE 2

Activity of L and DL isomers of amino acids

Amino acid	Assay organism	Activity of DL versus L isomers
		%
Histidine	*L. mesenteroides P-60*	51
Isoleucine	*L. mesenteroides P-60*	48
Lysine	*L. mesenteroides P-60*	46
Phenylalanine	*L. mesenteroides P-60*	51
Leucine	*L. arabinosus 17-5*	57
Valine	*L. arabinosus 17-5*	61
Methionine	*S. faecales R*	51
Threonine	*S. faecales R*	51
Tryptophan	*S. faecales R*	50

SUMMARY

The essential amino acids in fresh and dried blackeye peas, broccoli heads and stalks, spinach, potatoes, sweet potatoes, pinto beans, and raw and roasted peanuts were determined. Values for the 10 essential amino acids are presented on a fresh weight, dry weight, and protein basis for these vegetables.

Using the amino acid content of whole egg protein as a criterion, isoleucine, leucine, and methione are the amino acids which most often are limiting factors in the vegetables assayed.

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SURVIVAL AND MULTIPLICATION OF *MICROCOCCUS PYOGENES* VAR. *AUREUS* IN CREAMED CHICKEN UNDER VARIOUS HOLDING, STORAGE, AND DEFROSTING CONDITIONS

ROBERT P. STRAKA AND FRANCES M. COMBES
Western Regional Research Laboratory,[a] Albany, California

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Proctor and Phillips *(3, 4)* and Buchbinder, Loughlin, Walter, and Dangler (1) have discussed the possible health hazards of precooked frozen foods.

In 1946 Proctor and Phillips *(3)* conducted a survey on more than 70 different types of frozen precooked foods from 30 manufacturing establishments located in 11 states. The products were all purchased from retail vendors in their original containers, which varied in type and in quality of protection that they provided. The investigators demonstrated that frozen precooked foods, when thawed and allowed to stand, may serve as excellent substrates for the growth of microorganisms, including some types of bacteria significant from a public-health standpoint. They also noted that not all frozen precooked food products being manufactured and sold at that time were subject to either constant or strict bacteriological control.

Buchbinder *et al. (1)* of the New York City Health Department undertook a study, published in 1949, to determine whether potential health hazards exist in frozen precooked foods. They obtained samples from retail and occasionally from wholesale outlets. Chicken *a la king* was studied regularly after it became evident that this product was, in a sense, a special case. It became evident early in their study that frozen chicken *a la king* was yielding bacterial counts in excess of what might have been expected from the reports of Proctor and Phillips *(3, 4)*. More frequent testing of these products was then instituted and samples were collected over a period of one year. More than half of the 39 samples yielded a plate count in excess of 1,000,000 per gram. It was likewise found that 12 of the 39 samples yielded staphylococci of the food poisoning type. Eight of these gave staphylococcus counts of between 1,000 and 100,000 per gram, two gave counts between 100,000 and 400,000 per gram, and one gave a count of 2,000,000. In their summary, the authors stated that some samples of chicken *a la king* yielded plate counts and coliform counts which were higher than reasonable standards would allow. They applied a similar judgment to the finding of staphylococci of the food poisoning type and enterococci, each in about one-fourth of the chicken *a la king* samples examined.

In contrast with previous studies, which generally have been concerned with the occurrence of microorganisms, the present study deals specifically

[a] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

with the effect of prefreezing delay and various defrosting times on the survival and multiplication of *Micrococcus pyogenes* var. *aureus* in experimental samples of creamed chicken.

EXPERIMENTAL PROCEDURE

The creamed chicken was weighed into previously sterilized tin cans, size 211 x 200. Twenty-five grams of diced cooked chicken and 75 grams of white sauce (made up with skim milk, chicken broth, Crisco,[b] chicken fat, flour, and salt) were added to each of 250 containers. Some of the cans were inoculated with a food poisoning strain of *M. pyogenes* var. *aureus* obtained from Dr. R. V. Stone of the Los Angeles Board of Health. Other cans served as uninoculated controls. After sealing, certain containers were immediately placed in the −30°F.(−34.4°C.) freezing room, some were held at 77°F.(25°C.) for two hours before they were placed in the freezing room, and others were held for 5 hours at 77°F.(25°C.) before being placed in the freezer.

Samples were stored in the freezer for 2 days, 2 weeks, 4 weeks, 3, 6, and 12 months. After removal from the freezer, some samples were defrosted immediately in warm running water and aerobic bacterial plate counts were determined; certain samples were allowed to defrost at 77°F.(25°C.) for 6 hours, some for 10-11 hours at 77°F.(25°C.), and others for 18 hours at 77°F.(25°C.).

Before opening with a sterile can opener, the container was wiped with 95% alcohol and flamed with a Bunsen burner. The entire contents of the can (100 grams) were emptied into a 500-ml. screw-cap, sterilized blender jar, 400 grams of water were added, and the mixture was blended for 2 minutes at high speed. Serial aqueous dilutions in tryptose agar plates were incubated at 98.6°F.(37°C.) for 48 hours and counts determined.

RESULTS AND DISCUSSION

It is perhaps worth noting here that the conditions as set up in the experiment are somewhat arbitrary and the results are, of course, illustrative of what might be encountered in commercial practice. Significant factors which would conceivably alter the results are the size of package, freezing rate, variation in room temperature, frozen storage temperatures, and perhaps other conditions.

In order to interpret the data presented, one should keep in mind the temperature conditions encountered. Thus the prefreezing range of temperature in the contents of the containers changed from about 140-80°F. (60-26.7°C.) over a 5-hour period. When cans were thawed at room temperature of 75-77°F.(23.9-25°C.), it took about 1½ hours for the contents to reach a temperature of 34°F.(1.1°C.), 2 hours to reach a temperature of 48°F.(8.9°C.), and about 5 hours to reach a temperature of 70°F. (21°C.). The samples in frozen storage were held at −30°F.(−34°C.) where the killing effect of freezing probably would not be as marked as at higher freezing temperatures. The results are shown in Tables 1, 2, 3, and 4.

Since the initial temperature of the fill and subsequent cooling of the contents when the cans are allowed to stand at room temperature for various periods up to 5 hours might affect the bacterial content, a temperature record (Table 1) of 10 cans of uninoculated creamed turkey was made. Each can had a ¾-inch hole punched into the lid into which was inserted a No. 3 rubber stopper containing a thermometer. The lids were not sealed. Readings were taken at various intervals up to 5 hours. Since no diced

[b] Mention of this or other products does not imply that they are endorsed or recommended by the Department of Agriculture over others not mentioned.

TABLE 1

Cooling temperature record of creamed turkey when allowed to stand at room temperature (78°-80°F.) in tin cans (211 x 200) for various periods

Time	Temperature[c]	
	Average of 10 cans	Minimum and maximum
minutes	°F.	°F.
5	120	116-129
15	115	113-118
30	108	105-111
45	102	100-104
60	98	96-100
75	95	95-96
90	93	91-95
120	89	87-91
180	85	84-86
300	82	80-84

[c] Room temperature on recording thermometer varied between 78-80°F.(25.6-26.7°C.) from 10:00 a.m. to 6:00 p.m.

chicken was available for this particular experiment, diced turkey was substituted. It took approximately 1 hour for the creamed turkey to reach 98-100°F.(36.7-37.8°C.). Bergey's Manual lists the optimum temperature for the growth of *Micrococcus pyogenes* var. *albus* as 98.6°F.(37°C.).

When cans of frozen creamed chicken or turkey are held at room temperature, the material gradually reaches a temperature which permits microbial growth. To determine the time and temperature over a 24-hour period, a thawing temperature record (Table 2) was made of 11 cans of

TABLE 2

Thawing temperature record of frozen creamed turkey when removed from −30°F. (−34.4°C.) and allowed to stand at room temperature [68-77°F.(20-25°C.)] in tin can (211 x 200) for various times up to 24 hours

Time after removal from freezer	Temperature[d]		
	Room temperature	Average of 11 cans	Minimum-maximum
hours	°F.	°F.	°F.
¼	75	25	23-27
½	75	27	25-28
1	75	30	28-39
1½	77	34	28-48
2	75	48	41-54
2½	75	57	54-59
3	77	61	59-63
4	77	66	64-70
5	77	70	68-72
6	75	72	68-73
7	77	72	72-73
8	77	73	73-75
10	75	73	73-75
15	72	75	73-75
20	68	73	72-73
24	75	72	72-73

[d] Recorded by means of thermocouples by electron potentiometer.

frozen uninoculated creamed turkey (no diced chicken was available). The cans were held at −30°F.(−34.4°C.) for 2 weeks before they were placed at room temperature to thaw. The temperatures were recorded on a chart with an electronic potentiometer by means of copper and constantan thermocouples (B&S gauge No. 31) inserted through holes in the lids into the contents of the can. Before the cans were placed in the freezer the thermocouples were sealed on the lids with sealing wax. Table 2 shows the average temperatures of the 11 cans and also the minimum and maximum temperatures.

TABLE 3

Effect of delay in freezing, time of frozen storage, and elapsed time during defrosting, on increase in aerobic plate counts of uninoculated[a] precooked frozen creamed chicken

Time of frozen storage at −30°F.(−34°C.)	Ratio of final to initial aerobic plate counts for samples defrosted and held at 77°F.(25°C.)			
	Control[b]	6 hrs.	11 hrs.	18 hrs.
	Samples placed in freezer immediately after preparation			
2 days	2.5	7.2	.....	386
14 days	3.0	5.6	.....	227
28 days	2.8	2.0	.....	21
3 months	2.4	4.6	6.7	.....
6 months	2.5	2.7	6.0	.....
12 months	2.7	3.7	28.0[c]	.....
	Samples held at 77°F.(25°C.) for 2 hours after preparation before placing in freezer			
2 days	24	29	.....	1170
14 days	16	41	.....	1497
28 days	19	14	.....	81
3 months	26	14	36	.....
6 months	12	11	47	.....
12 months	13	14	206[c]	.....
	Samples held at 77°F.(25°C.) for 5 hours after preparation before placing in freezer			
2 days	181	322	.....	3595
14 days	154	215	.....	7241
28 days	265	221	.....	550
3 months	121	181	173	.....
6 months	153	162	163	.....
12 months	167	170	1547[c]	.....

[a] Initial count, 8,500 per ml. based on average of 2 containers.
[b] Defrosted immediately in warm running water.
[c] Room temperature went up to 86°F.(30°C.).

Table 3 shows the effect of delay in freezing, time of frozen storage, and elapsed time during defrosting on the increase in the aerobic plate counts of uninoculated precooked frozen creamed chicken. The results are expressed as the ratio of the final to the initial aerobic counts for samples defrosted and held at 25°C.(77°F.) for 6, 11, and 18 hours. Table 4 gives similar results for containers inoculated with *Micrococcus pyogenes* var. *aureus*.

Uninoculated Containers. The initial count was 8,500 per ml. and the figures given in Table 3 are averages of duplicate containers. No attempt

was made to identify the various colonies on the plates.

Table 3 gives the results for containers placed immediately in the —30°F.(—34.4°C.) freezer after sealing, held at this temperature for various periods from 2 days to 1 year, then removed and examined immediately, or after defrosting at 77°F.(25°C.) for 6, 11, or 18 hours. Eighteen hours were used in the first part of the experiment (2 to 28 days), but since the counts were very high, this period was shortened to 11 hours for the 3, 6, and 12 months' samples.

Table 3 gives similar results for containers held at 77°F.(25°C.) for 2 hours and also containers held for 5 hours before being placed in the freezer.

Uninoculated containers of creamed chicken placed in the —30°F. (—34°C.) freezer immediately after the cans were sealed. The count in the containers defrosted immediately (Table 3) in warm running water did not vary a great deal during 1 year of frozen storage. The slight increase of 1½- to 2-fold over the initial count probably occurred before freezing during the time it took for the samples to cool and freeze.

The containers defrosted in air at 77°F.(25°C.) for 6 hours after removal from the freezer (Table 3) gave increases which varied from 1- to 6-fold. These counts are a little higher than those obtained in the samples that were immediately defrosted. From Table 2 one can see that it took about 1½ hours for the can contents to reach 34°F.(1°C.), 2 hours to reach 48°F.(9°C.), and 6 hours to reach 72°F.(22°C.). So a very slight increase in the count would be expected. With a higher room temperature for the defrosting, the count probably would have been correspondingly higher.

In the containers defrosted and held in air at 77°F.(25°C.) for 11 hours (Table 3), there was a 5- to 6-fold increase in bacterial counts in the 3 and 6 months' storage samples, which is just a little greater than the increases recorded with samples defrosted and held for 6 hours. The 1-year storage samples were defrosted at a room temperature of 30°C. instead of 25°C. and this possibly is the reason for the considerably greater increase in count (27-fold) in this case. This was noted for all the containers defrosted on this particular day (Tables 3 and 4).

In those uninoculated containers defrosted in air at 77°F.(25°C) for 18 hours (Table 3), the 2-day frozen storage samples showed a 385-fold increase, the 14-day storage samples a 226-fold increase, and the 28-day storage samples a 20-fold increase. There was a considerable drop in bacterial count between the 14th and the 28th day of storage at 30°F. This same phenomenon was noted for all the samples defrosted at this time (Tables 3 and 4), and seems to indicate that the lethal effect of freezing between the 14th and 28th day is very pronounced.

Uninoculated containers of creamed chicken placed in the —30°F. (—34°C.) freezer after being held at 77°F.(25°C.) for 2 hours. The count in the containers defrosted immediately (Table 3) in warm running water after removal from freezing storage varied from a 11-fold to a 25-fold increase over a period of 1 year. This was a 5- to 9-fold increase over similar samples which had been placed in the freezer immediately (Table 3).

With the exception of the 14-day frozen storage sample, those contain-

TABLE 4

Effect of delay in freezing, time of frozen storage, and elapsed time during defrosting on increase in aerobic plate counts of precooked frozen creamed chicken inoculated with Micrococcus pyogenes var. aureus[b]

Time of frozen storage at −30°F.(−34°C.)	Ratio of final to initial aerobic plate counts for samples defrosted and held at 77°F.(25°C.)			
	Control[i]	6 hrs.	11 hrs.	18 hrs.
	Samples placed in freezer immediately after preparation			
2 days	1.2	1.3	.....	66.0
14 days	1.1	1.3	.....	19.0
28 days	1.2	1.0	.....	1.0
3 months	1.2	1.3	1.2	.....
6 months	1.4	1.1	1.7	.....
12 months	1.2	1.1	24.0[j]	.....
	Samples held at 25°C.(77°F.) for 2 hours after preparation before placing in freezer			
2 days	4.1	16	.....	66
14 days	2.5	4	.....	420
28 days	4.9	3	.....	12
3 months	3.6	10	13	.....
6 months	3.2	3	15	.....
12 months	2.8	3	175[j]	.....
	Samples held at 77°F.(25°C.) for 5 hours after preparation before placing in freezer			
2 days	32	66	.....	66
14 days	29	52	.....	607
28 days	35	42	.....	231
3 months	56	59	110	.....
6 months	26	48	202	.....
12 months	31	28	1035[j]	.....

[b] Initial count 370,000 per ml. based on the average of 2 containers.
[i] Defrosted immediately in warm running water.
[j] Room temperature went up to 86°F.(30°F.).

ers defrosted at 77°F.(25°C.) for 6 hours after removal from freezing storage (Table 3) gave counts which were not much different from those in the containers defrosted immediately.

In the samples defrosted for 11 hours at 77°F.(25°C.) there was about a 2- to 4-fold increase in counts in the 3 and 6 months' samples over similar samples defrosted for 6 hours. The 1-year storage samples defrosted for 11 hours at 30°C. gave about a 13-fold increase over similar samples defrosted for 6 hours (Table 3). In this case, as previously explained, the room temperature on this particular day was 86°F.(30°C.), thus probably accounting for the higher count.

The samples defrosted at 77°F.(25°C.) for 18 hours (Table 3) gave counts which ranged from 4- to 40-fold higher than similar samples defrosted for 6 hours under similar conditions. Obviously, 18 hours is too long to defrost samples which had been held for 2 hours prior to being placed in the freezer. Even defrosting similar samples for 11 hours is too long.

Uninoculated containers of creamed chicken placed in the −30°F.(−34°C.) freezer after being held at 77°F.(25°C.) for 5 hours. All of

these containers (Table 3), whether defrosted immediately or for 6, 11, or 18 hours, gave enormous increases in the counts. Holding samples for 5 hours before placing them in the freezer gives time for a large bacterial build-up, which is bound to adversely affect the quality of the product.

Inoculated containers. These containers were inoculated with a food poisoning strain of *M. pyogenes* var. *aureus* and the original total count was 378,000 per ml. The figures given in Table 4 are the averages of duplicate containers. The initial count may seem a little high, but not in the light of results obtained in commercial packages by Buchbinder *et al. (1)*. *M. pyogenes* var. *aureus* colonies produced typical yellow colonies on tryptose agar plates.

The inoculated containers were treated and handled in much the same manner as the uninoculated containers discussed previously.

Inoculated containers of creamed chicken placed in the −30°F.(−34°C.) freezer immediately after the cans were sealed. The counts in the containers defrosted immediately (Table 4) in warm running water varied very little over a period of a year, being only $\frac{1}{10}$- to $\frac{4}{10}$-fold higher than the original count.

Those containers defrosted in the air at 77°F.(25°C.) for 6 hours likewise showed very little increase after having been in the freezer over a period of 1 year. The increase ranged from none to $\frac{3}{10}$-fold.

In the samples defrosted for 11 hours (Table 4) at 77°F.(25°C.) there was about a $\frac{2}{10}$- and $\frac{7}{10}$-fold increase for the 3 and 6 months' samples, respectively. The 1-year samples gave about a 23-fold increase. As previously explained with the uninoculated samples, the room temperature on this particular day rose to 86°F.(30°C.), thus probably accounting for the accelerated increase in bacterial numbers.

The samples defrosted at 77°F.(25°C.) for 18 hours after having been held at −30°F.(−34°C.) for 2 days gave a 65-fold increase, the 14-day frozen samples gave an 18-fold increase, and the samples which had been frozen for 28 days showed no increase. As indicated with the uninoculated containers, we do not know why the 28-day frozen storage samples did not increase in numbers when placed at room temperature for 18 hours. Again, it may be that the lethal effect of freezing between the 14th and 28th day is very pronounced.

Inoculated containers of creamed chicken placed in the −30°F.(−34°C.) freezer after being held at 77°F.(25°C.) for two hours. In the containers defrosted immediately (Table 4) in warm running water there was a 1½- to 4-fold increase over samples which had been placed in the freezer immediately (Table 4).

In those containers defrosted at room temperature [77°F.(25°C.)] for 6 hours the 2-day frozen storage samples gave a 15-fold increase over the original count; however, the 14-day and 28-day storage samples gave only a 3- and a 2-fold increase, respectively. The 3 months' samples gave a 9-fold increase but the 6 and 12 months' samples showed only a 2-fold increase.

With 11 hours of defrosting at 77°F.(25°C.), the 3 and 6 months' frozen storage samples gave 12- and 14-fold increases in the counts. The

1-year samples gave a 174-fold increase, again probably due to the elevated defrosting temperature as previously explained.

With 18 hours of defrosting at 77°F.(25°C.), the 2-day frozen storage samples gave a 65-fold increase, the 14-day samples a 419-fold increase, and the 28-day samples an 11-fold increase. Again, as previously noted, there was an enormous drop in count between the 14-day and 28-day frozen storage samples.

Inoculated containers of creamed chicken placed in the −30°F.(−34°C.) freezer after being held at 77°F.(25°C.) for 5 hours. All of these containers (Table 4), whether defrosted immediately or for 6, 11, or 18 hours, gave large increases in counts. Holding samples for 5 hours at room temperature [77°F.(25°C.)] before placing them in the freezer gives time for a large bacterial build-up, which is bound to adversely affect the quality of the product.

SUMMARY

Under the conditions of our experiment, the results indicate: (a) containers of creamed chicken should not be held for more than 2 hours before being placed in the freezer; and (b) quickly frozen samples can be defrosted at 77°F.(25°C.) over a 10- to 11-hour period without encountering excessive counts (60,000 per gram for uninoculated containers and about 600,000 for containers initially inoculated with about 370,000 organisms per gram).

The freezing without delay is more important (as expected) than thawing without delay. The relative margins of 2 hours before freezing and of 11 hours before use of the thawing products, are useful guides, but, of course, would differ under markedly different conditions.

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VARIATION IN HYDROGEN ION CONCENTRATION AND TOTAL ACIDITY IN VINEGAR

TREVA C. KINTNER AND MARGARET MANGEL
Department of Home Economics, University of Missouri, Columbia, Missouri

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In connection with research on cooked salad dressing, variation in the pH of the final product was shown to be related to the pH of the vinegar used. Considerable variation in pH was found in the same type of vinegar and even in that bottled under the same brand name. Most reports on vinegar in current literature are based on research completed as long as 50 years ago. More data are available on total acidity, which is controlled by law, than on pH. Figures for the latter are usually given as averages with no information on variation among types and brands of vinegars. The present study was made to obtain a range of values for pH and percent acetic acid for types of vinegar on the current retail market. Of these, cider or apple vinegar is the most widely used. Vinegars have also been made from dilute ethyl alcohol, malt infusion, sugar, molasses, grapes, oranges, bananas, honey, dates, raisins, pears, apricots, peaches, and pineapple. The vinegar which is cheapest and most often used by commercial industries is the distilled or spirit vinegar which usually contains a high percent of acetic acid. A distilled sugar or acid vinegar, with caramel flavoring added, comes in open barrels and is available in states which have no laws opposing the sale of compounded vinegars. Other flavorings as garlic, eschalot, mint, basil, tarragon, dill, thyme, marjoram, or herb salad are found in wine vinegars while tarragon is often added to malt vinegars. The definitions of vinegar, its uses, methods of preparation and analyses have been reviewed by Brannt *(3)*, Jacobs *(4)*, Joslyn *(5)*, Triebold *(6)*, Wetherill *(8)*, and Woodman *(10)*.

EXPERIMENTAL PROCEDURE

The hydrogen ion concentration of the vinegar was measured electrometrically by the glass electrode at 25°C. on the Beckman pH meter. The total acidity was measured as recommended by the Association of Official Agricultural Chemists *(2)* and computed and reported as percent by weight of acetic acid. The acid neutralized during production of the vinegar was estimated as acetate by substitution of the experimentally determined pH and total acidity into the Henderson-Hasselbalch equation.

RESULTS AND DISCUSSION

Table 1 shows the hydrogen ion concentration and the total titratable acidity of all of the vinegars studied. The mean pH of all of the vinegars was 3.05, with a range of 2.55 to 3.50. This mean is comparable to average pH's, 3.0 and 2.9, reported by West *(7)* and the American Home Economics Association *(1)*. Most of the bottled cider vinegars, with a mean pH of 3.25, and a range of 3.07-3.50 had a lower hydrogen ion concentration than other kinds tested. The variation in pH of open barrel cider vinegar was greater than for bottled, as shown by a pH range of 2.85 to 3.30 for 8

samples, and the mean pH, 3.08, was lower. The mean pH of white wine vinegar was 3.00, while that of the red wine was more acid at 2.87. These vinegars were quite uniform in hydrogen ion concentration. Malt, peach, pear, and apricot vinegars had pH's similar to bottled cider vinegar. The distilled vinegars were the most acid of the vinegars tested, with a mean pH of 2.74 and a range for 8 samples of 2.55 to 2.90. The distilled sugar had a higher mean pH at 2.80 than the regular distilled at 2.64.

TABLE 1

pH and percent acetic acid of vinegars

Type of vinegar	Description	pH			Acetic acid		
		Number of samples	Mean	Range	Number of samples	Mean	Range
						%	%
Cider	Bottled	18	3.25	3.07-3.50	9	4.4	3.9-5.0
	Open barrel	8	3.08	2.85-3.30	5	4.4	3.8-5.0
	All cider	26	3.19	2.85-3.50	14	4.4	3.8-5.0
Wine	Red bottled	6	2.87	2.80-2.97	6	5.1	4.7-5.5
	White bottled	3	3.00	2.96-3.04	3	4.9	4.7-5.0
	All wine	9	2.91	2.80-3.04	3	5.0	4.7-5.5
Malt	Bottled	3	3.07	3.02-3.10	3	5.3	5.2-5.6
Peach	Bottled	1	2.98	.....	1	5.4	.....
Pear	Bottled	1	3.25	.....	1	4.4	.....
Apricot	Bottled	1	3.10	.....	1	5.0	.....
Distilled	Regular bottled	5	2.64	2.55-2.80	4	5.1	5.0-5.2
	Sugar open barrel	3	2.80	2.65-2.90	2	3.6	3.5-3.7
	All distilled	8	2.74	2.55-2.90	6	4.6	3.5-5.2
All vinegars		49	3.05	2.55-3.50	35	4.7	3.5-5.6

A minimum of 4 g. of acetic acid in 100 ml., or an acetic acid content of 4%, is required by law. The mean per cent acetic acid for 35 samples tested was 4.7, with a range of from 3.5 to 5.6. These values exclude two samples of wine vinegars, with 6.4% acetic acid, which had been stored for several years. A mean percent acetic acid for all cider vinegars tested was 4.4, with a range of 3.8 to 5.0. Winton and Winton *(9)* reported the percent acetic acid for cider vinegars at 4.84. There was little difference in the mean total acidity of bottled and open barrel cider vinegars. The red wine was similar in total acidity to the white wine vinegars with a mean of 5.0 and a range of 4.7 to 5.5%. This mean is considerably lower than that of 5.57 reported by Winton and Winton. The samples of malt vinegar averaged 5.3% acetic acid in contrast to the 4.23 reported by the above mentioned authors. The peach, apricot, and pear vinegars showed total acidities of 5.4, 5.0, and 4.4%, respectively. The distilled vinegars tested showed a range in percent acetic acid from 3.5 to 5.2, with a mean of 4.6. The total acidity of the bottled distilled vinegars was between

5.0 and 5.2%, but that for distilled sugar vinegar, 3.5 to 3.7, was lower than the legal limit. The difference between the mean total acidity, 5.1%, of bottled distilled vinegar and that of 6.34% reported by Winton and Winton, is probably due to an attempt by the manufacturer to produce a uniform 5% bottled distilled vinegar.

A preliminary examination of the relationship between total acidity and pH was made, using scatter diagrams. When the mean values of the different types of vinegar were compared, the relationship was similar for all types except for the sugar distilled vinegar. When the values for the individual samples were plotted, the pH of all distilled vinegars was lower in relation to total acidity than those of fruit and malt vinegars. Open barrel vinegars, both the sugar distilled and cider, were more variable and showed less consistent relationship between pH and total acidity than the other vinegars.

An examination of the relationship between total acidity and pH by substitution of these values into the Henderson-Hasselbalch equation indicated a greater neutralization of acid in those vinegars made from fruits than in distilled type vinegars. The estimated acetate in terms of millimolar concentration is shown in Table 2. The mean acetate concentration

TABLE 2

Concentration of acetate in vinegars as estimated by substitution into Henderson-Hasselbalch equation

Type of vinegar	Description	Number of samples	Acetate in millimoles	
			Mean	Range
Cider	Bottled	6	19.6	15.3-24.9
	Open barrel	5	16.2	8.6-28.9
	All cider	11	18.1	8.6-28.9
Wine	Red	6	10.9	9.2-12.7
	White	3	14.5	13.0-15.9
	All wine	9	12.1	9.2-15.9
Malt	Bottled	3	18.2	17.0-19.0
Peach	Bottled	1	14.9	.....
Pear	Bottled	1	22.5	.....
Apricot	Bottled	1	18.2	.....
Distilled	Regular bottled	4	5.9	5.2-7.2
	Sugar open barrel	2	6.4	4.8-8.0
	All distilled	6	6.1	4.8-8.0
All vinegars		32	14.2	4.8-28.9

for all vinegars was 14.2, with a range of from 4.8 to 28.9 millimoles. The acetate estimate for cider, wine, and other fruit and malt vinegars ranged from 8.6 to 28.9 millimoles, while the distilled was considerably lower with a range of from 4.8 to 8.0 and a mean of 6.1 millimoles.

SUMMARY

A survey of the literature indicated a need for more information on pH and total acidity of the many types of vinegar available at the present time. The mean pH of 49 samples of 7 different types of vinegars was 3.05. The pH values for the individual samples ranged from 2.55 to 3.50. The percent of acetic acid for 35 of these samples ranged from 3.5 to 5.6 with a mean of 4.7%. The low pH's obtained in relation to total acidity for distilled vinegars might be explained by the low salt formation in vinegar of this type.

The range in both pH and total acidity obtained in this study is smaller than that reported previously, which may indicate a trend toward uniformity in vinegars on the market today.

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AN ELECTRONIC METHOD FOR CONTINUOUS DETERMINATION OF RAPID TEMPERATURE CHANGES IN THERMAL DEATH-TIME STUDIES[a]

JOSEPH A. STERN, MELVIN A. HERLIN, AND BERNARD E. PROCTOR
Departments of Food Technology and Physics, Massachusetts Institute of Technology, Cambridge, Massachusetts

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The thermal resistance of spores of spoilage organisms and the rate at which heat penetrates into food products are important factors in determining the degree and duration of heat to be applied to canned foods for the purpose of preservation. Early investigators in the field of thermal death-time studies assumed correction times for lag in heat penetration *(3, 4, 10)*. Ball, Merrill, Williams, and Wessel *(2)* measured heat penetration in thermal death-time tubes and arrived at correction times for individual foods under particular conditions. Previous to the investigation of Sognefest and Benjamin *(8)*, most of the correction factors proposed for lag in heat penetration were small and practically negligible when compared with the thermal death time (of the order of 10 minutes). However, these latter investigators demonstrated that in thermal death-time tests of relatively short duration (1 minute to 3 minutes) the lag and the come-up times become an appreciable percentage of the total thermal death time and that proper correction factors result in lower F and z values[b] than when instantaneous heating and cooling are assumed without applying the corrections.

Stumbo *(9)* used extremely small samples of food in his thermal death-time studies but was unable to determine experimentally the heating and cooling rates of the samples because of the short time intervals involved. Instead, the heating and cooling curves were calculated by the mathematical method of Olson and Schultz *(7)*. The sample was considered to be an infinite slab of material, and any lag effects ascribable to the container were considered to be negligible.

The purpose of the present study was to develop a continuously recording device that would accurately measure and record large temperature changes in foods and other materials in short periods of time [from 15° to 400°F.(−9.5° to 204.5°C.) per second] and to compare actual come-up curves with those calculated mathematically. It was believed that an entirely electronic device would be best for the measurement of rapid temperature changes. The recorder described in detail here was developed independently by Luyet and Gonzales *(5)*, who described and demonstrated the use of an almost identical device for the measurement of ultra-rapid temperature

[a] Contribution No. 134 from the Department of Food Technology, Massachusetts Institute of Technology.

[b] According to the nomenclature of Ball *(1)*, F equals the number of minutes required to destroy the organism (in any specific medium) at 250°F.(121.1°C.), and z equals the slope of the straight-line semi-logarithmic thermal death-time curve (that is, the number of degrees Fahrenheit required for the curve to pass through one logarithmic cycle).

changes in materials during freezing processes. Our apparatus differs primarily from theirs in the choice of the amplifier and in the method of calibration of the recorder.

DESCRIPTION OF APPARATUS

The apparatus consists, in the main, of a copper-constantan thermocouple, a DC amplifier, a cathode ray oscilloscope, a resistor-condenser (RC) circuit, and a standard 35-mm. camera as the recording device.

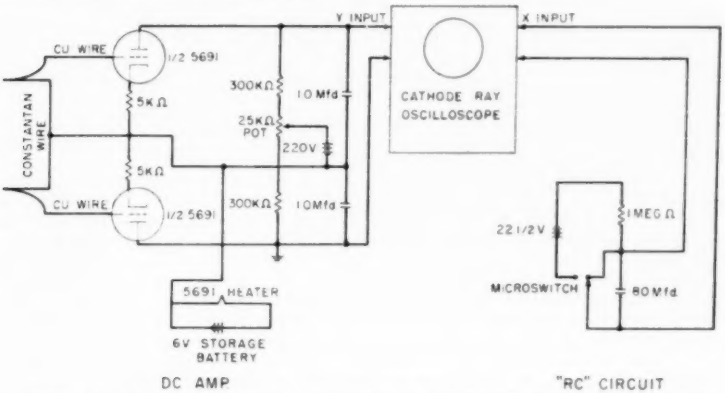

Figure 1. Schematic diagram of apparatus for continuous electronic measurement of rapid temperature changes.

A schematic diagram of the apparatus is presented in Figure 1. The sequence of operation is such that the electromotive force (e.m.f.) induced in the thermocouple is fed into the amplifier and then into the Y-input of the oscilloscope.

The DC amplifier is a balanced one-tube circuit, in which an RCA Red Base 5691 tube is used for maximum stability. The amplifier was designed specifically for the purpose of amplifying the exceedingly small voltages generally encountered in the use of thermocouples to voltages sufficient to effect a vertical deflection of the cathode ray beam.

The cathode ray oscilloscope is similar in many ways to a television set but has only one sweep or line across the fluorescent screen. Actually the line is a dot moving so rapidly across the face of the cathode ray tube that it appears as a line, because of the persistence of the image of the dot on the screen and on the retina of the eye. By use of a specially designed RC circuit, it is possible to slow down and control the horizontal speed of the dot so that it will take 5, 10, 15, or 20 seconds to travel the 4 inches across the face of the cathode ray tube.

The amplified signal received by the oscilloscope causes the dot to move in a vertical direction. The greater the temperature difference between the two junctions of the thermocouple, the greater the deflection of the dot from the position that it occupies when no voltage is induced in the thermocouple (that is, when the junctions are at the same temperature). When the apparatus is in operation, the dot therefore traces a path across the face of the tube. This path serves as a continuous measurement of the change in temperature of the material under study with reference to time.

The attachment to the apparatus of a camera with a fixed focus makes it possible for the operator to obtain a permanent record of the curve while simultaneously observing the cathode ray screen. A time exposure is required. A standard 35-mm. camera has been used in our experiments to date, but plans are underway to use a Land camera in order to avoid delay in the development of the film.

CALIBRATION OF CATHODE RAY SCREEN

The cathode ray oscilloscope is generally equipped with a plastic or glass plate located in front of the tube screen. Cross-section markings on this plate serve as a satisfactory calibration of the screen for rough approximations. These markings are unsatisfactory for accurate work, however, because the response of the thermocouple throughout the temperature range under investigation may not be linear and, in addition, because the screen of the cathode ray tube is actually a portion of a sphere and is slightly curved. Therefore, it is necessary to calibrate the screen.

The calibration is accomplished in the following manner:

Temperature scale (vertical or Y scale). The reference temperature junction of the thermocouple is maintained in an ice-and-water bath, and the measuring junction is immersed in various solutions of known temperature. At each reference temperature chosen, the lens of the camera is opened and the cathode ray beam swept across the screen. This results in a series of horizontal lines on the film, each representing a known temperature.

Time scale (horizontal or X scale). After the speed of the cathode ray beam is adjusted to give the desired sweep time, the dot is brought to the center of the screen by adjustment of the "Up-Down" dial of the oscilloscope and then moved as far to the left as desired by adjustment of the "Left-Right" dial. The camera lens is then opened (on the same film as used for the temperature scale) and the dot swept up and down by twisting the "Up-Down" dial to give the zero time line. This is repeated at various points along the horizontal diameter of the screen at known time intervals.

The above two procedures result in a film with both temperature and time reference lines, namely, a calibration film.

The negative of the calibration film is superimposed upon the negative of the actual temperature-time curve obtained in any given test, and from these negatives a photographic print is obtained that shows both the actual curve and the reference lines. For proper superimposition of the negatives it has been found advisable to sweep two reference lines onto the film before determination of the temperature-time curve. The zero-time line is swept first, and instead of sweeping a temperature reference line, the dot is permitted to travel a short distance across the screen at the starting temperature previous to the determination of the temperature-time curve.

RESULTS AND DISCUSSION

A typical record obtained with the apparatus described above is presented in Figure 2, which is a photograph of the temperature-time curve of nutrient broth. The procedure used was as follows: With the aid of a hypodermic syringe and a 2-inch, 28-gauge needle, a 0.01-ml. sample of nutrient broth was placed 2 inches below the top of a capillary tube, which was 4 inches in length. The lower end of the capillary tube had been sealed previously. The inner diameter of the tube was 0.8 mm. and the outer diameter, 1.5 mm. The resulting cylinder of nutrient broth was 15 mm. in height and 0.8 mm. in diameter. After the measuring junction of the thermocouple was inserted into the center of the sample, the opening of the tube was sealed with plasticene. The tube was then immersed in a hot oil bath (172°C.) and the record taken.

The purpose of placing the sample somewhere near the center of the capillary tube was to minimize heat flow into the sample from the ends of the tube, thereby approximating the infinite cylinder of Olson and Schultz *(7)*.

When the temperature-time data had been obtained experimentally, the f and k values[c] of the system were determined by methods described by

[c] Here f represents the time required for the straight line, semi-logarithmic, heat penetration curve to traverse one logarithmic cycle, and k represents the thermal diffusivity of the system.

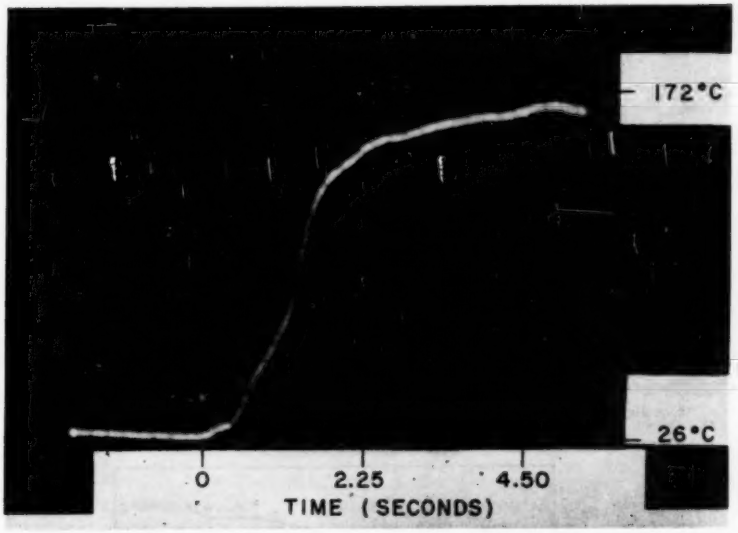

Figure 2. Temperature-time curve for a sample of nutrient broth, typical of curves obtained with the apparatus.

Olson and Jackson *(6)*. These values are: f is 2.58 seconds and k is 0.0869 mm.^{2} per second. A theoretical temperature-time curve for an infinite cylinder was then calculated, with the use of the formula given by Olson and Schultz and the k factor cited above. The results of this calculation are presented in Table 1. For comparison, the k values at different temperatures were calculated from the experimental curve, the formula of Olson and Schultz for an infinite cylinder again being used. The average value of k was found to be 0.0899, which is in fair agreement with that determined by the method of Olson and Jackson.

Figure 3 shows a tracing of the experimental curve and a plot of the mathematically derived curve. The curves are in excellent agreement, differing by a maximum of only 0.4 second in the time necessary to arrive at a given temperature.

The experimental temperature-time curve obtained by this method indicates that the sample treated, although liquid, was heated by conduction rather than by convection. A possible explanation for this may be that the gravitational force created by the change in density of the solution on heating was insufficient to overcome what is commonly called the "capillary force" of the tube. Consequently, the typical convection currents were unable to flow or be formed, and the sample was heated by conduction.

As a result of this work it appears that the mathematical method of Olson and Schultz may be applied to the calculation of come-up times of small samples of material with little error in the results, even though the thickness of the container wall may approach that of the sample. However,

TABLE 1

Comparison of experimentally obtained and theoretically calculated values of a temperature-time curve for a small sample of nutrient broth

Time	Experimental values				Theoretical values			
	T_t	$u=C(\theta)$	θ	k	T_t	$u=C(\theta)$	θ	k[a]
seconds	*°C.*			*mm.² per second*	*°C.*			*mm.² per second*
0.25	.....	.....	.....	.....	26.5	0.99682	0.0386	0.0869
0.50	.....	.....	.....	.....	36.5	0.92699	0.0772	0.0869
0.64	46.5	0.859	0.097	0.0904	46.5	0.85936	0.0982	0.0869
1.02	67.0	0.719	0.134	0.1030	79.0	0.63725	0.1574	0.0869
1.25	87.5	0.578	0.175	0.1073	95.7	0.52173	0.1930	0.0869
1.40	108.0	0.438	0.224	0.0859	106.0	0.45268	0.218	0.0869
1.59	128.5	0.298	0.291	0.0739	115.0	0.38782	0.245	0.0869
2.12	149.0	0.157	0.404	0.0787	137.5	0.24170	0.327	0.0869
3.60	163.8	0.0572	0.576	0.0900	162.9	0.0625	0.565	0.0869
4.00	.....	.....	.....	.....	165.6	0.0453	0.617	0.0869
Average				0.0899				

[a] According to Olson and Jackson, for an infinite cylinder $k = 0.398\ r^2/f = [0.398 \times (0.75)^2]/2.58 = 0.0869$ mm.² per second, where r is equal to the overall radius of the cylinder.

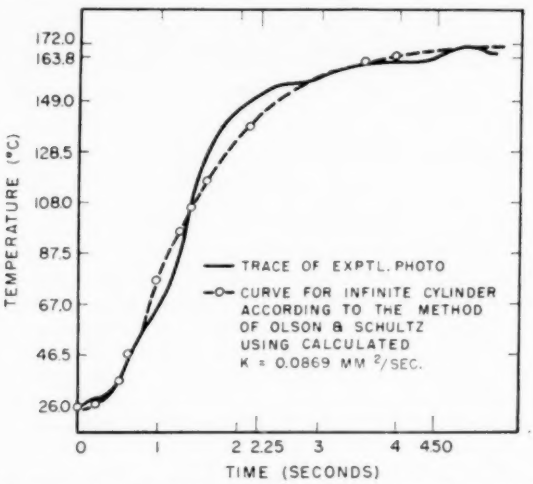

Figure 3. Comparison of temperature-time curves obtained experimentally and mathematically for a sample of nutrient broth.

the k of the system as an entity must be considered, although in the calculation of the mathematical curve the f value only of the system need be known. This is shown for an infinite cylinder by the equations below. The symbols used in these equations are those defined by Olson and Schultz.

$$k = (0.398\ r^2)/f \qquad \text{(Olson and Jackson)}$$

$$\left.\begin{aligned} u &= C(\theta) = (T_1 - T_t)/(T_1 - T_0) \\ \theta &= kt/r^2 \end{aligned}\right\} \text{(Olson and Schultz)}$$

Therefore:

$$\theta = (0.398\, r^2/f) \times (t/r^2) = 0.398\, t/f$$

and

$$u = C(0.398\, t/f).$$

From the above equations it appears that the diameter of the infinite cylinder does not affect the temperature. However, it must be remembered that the f value is directly affected by the thickness of the container walls and the overall dimensions of the cylinder.

Compared with the overall come-up time, the lag ascribable to heat penetration (which must be considered in systems of larger dimensions) appears to be of little importance in systems of extremely small size where the temperature changes are exceedingly rapid. However, it may be concluded that in the studies with hot oil baths the time necessary for the sample, even though small in amount, to come-up to temperature is considerable. Hence one should accurately measure and calculate the lethal values of the come-up temperatures rather than assume an instantaneous come-up time or a small correction factor.

SUMMARY

A continuously recording electronic device for measurement of rapid temperature changes within a system is briefly described, and the method of calibrating the device is explained.

A theoretical heating curve derived by the mathematical method of Olson and Schultz is shown to agree with an experimental curve obtained with the apparatus.

The use of the apparatus in thermal death-time studies is discussed briefly.

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